Chlorpyrifos Issue Paper:
Evaluation of Biomonitoring Data from Epidemiology Studies

EPA's Office of Pesticide Programs
March 11, 2016

Table of Contents

Table of Figures

Table of Tables

1.0 Introduction

Chlorpyrifos (*0,0*-diethyl-*0*-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum, chlorinated organophosphate (OP) insecticide, acaricide, and miticide used to control a variety of insects. It was first registered in 1965 for control of foliage and soil-borne insect pests on a variety of food and feed crops. Risk mitigation actions in 1997 and 2000 led to nearly all residential uses being voluntarily cancelled by Dow AgroSciences. Currently, registered uses include food and feed crops, golf course turf, greenhouses, non-structural wood treatments (such as utility poles and fence posts), ant bait stations, fire ant mounds, a USDA quarantine use for containerized/potted ornamentals, and use as an adult mosquitocide.

The agency has taken a stepwise, objective and transparent approach in evaluating, interpreting, and characterizing the strengths and uncertainties associated with all of the available lines of scientific information related to the human health effects of chlorpyrifos. Like other OPs, chlorpyrifos binds to and phosphorylates the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. This can lead to accumulation of acetylcholine and, ultimately, at sufficiently high doses, to clinical signs of toxicity. In addition to the AChE inhibiting potential of chlorpyrifos, there is a growing body of literature with laboratory animals (rats and mice) indicating that gestational and/or early postnatal exposure to chlorpyrifos may cause persistent effects into adulthood along with high quality epidemiology studies which have evaluated prenatal chlorpyrifos exposure in mother-infant pairs and reported associations with neurodevelopment outcomes in infants and children.

The stepwise evaluation began with the September 2008 FIFRA Scientific Advisory Panel (SAP) meeting involving a preliminary review of the literature for chlorpyrifos, with a particular focus on women and children (USEPA, 2008). In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of scientific evidence, including epidemiology, in the context of the understanding of the adverse outcome pathway (or mode of action (USEPA, 2010). The draft framework, which includes two key components: problem formulation and use of the modes of action/adverse outcome pathways (MOA/AOP) frameworks, was reviewed favorably by the SAP in 2010 (FIFRA SAP, 2010). OPP's draft framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework, which highlight the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek *et al.*, 2014).

In 2011, the agency released "Chlorpyrifos: Preliminary Human Health Risk Assessment for Registration Review," focusing on the AChE inhibiting potential of chlorpyrifos (USEPA, 2011) and included assessment of exposures from dietary (food, water), occupational and residential

pathways. This focus was consistent with the recommendation from the 2008 SAP that AChE data provide the most appropriate endpoint and dose-response data for deriving points of departure (PoDs) for purposes of risk assessment. Also in 2011, the physiologically based pharmacokinetic-pharmacodynamic model (PBPK-PD) was reviewed by the FIFRA SAP (FIFRA SAP, 2011).

In 2012, the agency convened another meeting of the FIFRA SAP on chlorpyrifos which incorporated the newest experimental data related to AChE inhibition and both cholinergic and non-cholinergic adverse outcomes, including neurodevelopmental studies on behavior and cognition effects (FIFRA SAP, 2012). Similarly, the agency also performed a more in-depth analysis of the biomonitoring data and of epidemiological studies from three major children's health epidemiology cohort studies in the U.S., as well as developed plausible hypotheses on MOAs/AOPs leading to neurodevelopmental outcomes (USEPA, 2012a). Following the 2012 SAP meeting, the agency solicited additional input from federal experts in the areas of Magnetic Resonance Imaging (MRI) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological studies.[1] In 2012, the agency also conducted an assessment of spray drift exposure which led to label mitigation addressing risk concerns (USEPA, 2012b).

In December, 2014, the agency released "Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review" (herein called HHRA) which included the use of a PBPK-PD model (Appendix 2) model to derive human PoDs. This model obviated the need for the animal to human extrapolation factor, and refined intra-species factors for some lifestages (USEPA, 2014a). The chlorpyrifos 2014 revised HHRA also included retention of the 10X FQPA Safety Factor due to uncertainty regarding the degree of protection that the endpoint of AChE inhibition provides for potential neurodevelopmental effects (USEPA, 2014a).

In recent years, the National Academy of Sciences has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision making (NRC, 2011, 2014). EPA's Office of Chemical Safety and Pollution Prevention is currently developing systematic review policies and procedures. As part of the 2014 chlorpyrifos revised human health risk assessment and again in 2015 for all the OPs (USEPA, 2015), the agency has reviewed and updated the experimental toxicology literature search since the 2012 SAP using the concepts consistent with systematic review such as detailed tracking of search terms and which literature have been included or excluded. That systematic review is not repeated here but can be found in 2012 SAP issue paper, the 2014 revised HHRA, and updated for the 2015 literature review which supports risk assessments for all registered OPs.

[1] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170

Based on the systematic review of the literature, the 2014 human health risk assessment identified uncertainty in the degree to which points of departure derived from AChE inhibition are protective for neurodevelopmental effects in humans. It is this uncertainty which provides the foundation for retaining the 10X FQPA Safety Factor in 2014. The agency published a 2015 proposed tolerance revocation for chlorpyrifos based on human health risks identified in the 2014 HHRA. If the revocation action becomes effective as proposed, it would likely lead to the end of chlorpyrifos use on food crops or in processed food produced in or imported into the U.S. In the proposed tolerance revocation, the agency noted that the evaluation of the available information including biomonitoring was continuing—this biomonitoring evaluation is the focus of this paper. While EPA would have preferred to complete that analysis prior to commencing rulemaking, the timing for the proposal was directed by the U.S. Court of Appeals for the 9th Circuit, which ordered EPA to respond to an administrative petition to revoke all chlorpyrifos tolerances by October 31, 2015.

The agency acknowledges that in 2012, the SAP noted that there was "general agreement that the data from these [epidemiology] studies alone were not sufficient to derive a point of departure (POD) for purposes of quantitative risk assessment (p. 50, 2012 SAP report)." However, the Panel further "recognized the value of these data and urged the agency to find ways to use the epidemiology studies, and in particular, the data from the CCCEH [Columbia Center for Children's Environmental Health Mothers and Newborn Study] study, to inform the dose-response assessment of chlorpyrifos… if one assumes that cord blood measurements reflect exposure levels during the critical prenatal period for induction of neurodevelopmental effects, then in theory, these would be the ideal data from which to derive the POD for chlorpyrifos in humans." The Panel went on the state that "a [PBPK] model could be used to further characterize the dose estimates in the epidemiology studies, for additional dose response analyses. Such a PBPK model will become even more important in the event that the agency might, at some point in the future, decide to move from using AChE inhibition to another outcome." The agency has followed up on the SAP recommendations to use the PBPK model to further assess the epidemiological data. The status of the analysis of the biomonitoring evaluation is provided below. At this point in time, the agency is soliciting comment from the SAP on changing the critical effect for quantitative risk assessment from AChE inhibition to neurodevelopmental outcomes thereby changing the PoDs from doses eliciting 10% RBC AChE inhibition to adverse effects changes in neurodevelopment as measured by epidemiology studies conducted by CCCEH.

2.0 Scope & Content of this 2016 Issue Paper

As summarized in Section 1 above, the agency has a long history of detailed technical analysis of various aspects of chlorpyrifos hazard and exposure assessment. In order to focus this issue paper on the pertinent issues--namely the transition from using AChE inhibition to adverse neurodevelopmental effects with the epidemiology biomarker data from CCCEH as the critical

effect for hazard assessment—the details of these previous evaluations are not repeated in here. Instead, the agency encourages the Panel and the public to also read these two documents *before* reading this 2016 issue paper:

- USEPA (2014a). Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review, December 29, 2014, D424485;
- U.S. Environmental Protection Agency. (2015) Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides. September 15, 2015, D331251.

These two documents (and associated appendices) provide much of the scientific foundation for the current 2016 issue paper, and are available in the SAP public docket EPA-HQ-OPP-2016-0062 at www.regulations.gov. For example, these 2014 and 2015 documents summarize the systematic reviews for epidemiology and experimental toxicology studies which support the agency's conclusion that chlorpyrifos exposure results in long-term neurobehavioral effects and that chlorpyrifos contributed to the outcomes reported by the CCCEH researchers. It is noted that as part of these 2014 and 2015 systematic reviews, the agency has evaluated, and excluded from further consideration at this time, other critical effects including birth outcomes and autonomic nervous system impacts (see Appendix 3.0 for further details). The 2014 and 2015 documents also contain the agency's reviews of the epidemiology studies, detailed summary of the PBPK model, and detailed analysis of contemporary exposure from food, water, and occupational/residential exposure. Limited summary information most pertinent to this 2016 issue paper is contained in appendices here but for those individuals needing more details, refer to the citations above.

The remainder of the document is organized by the following:

- *Section 3.0 Brief Summary of CCCEH Findings and Maternal & Cord Blood Levels* provides a brief synopsis of the key findings of CCCEH regarding neurodevelopmental outcomes associated with chlorpyrifos exposure. This section also provides summary data provided by the CCCEH investigators of blood levels across time.

- *Section 4.0 Lifestages for Consideration* describes the relevant lifestages for assessing the neurodevelopmental effects as context for the remainder of the issue paper.

- *Section 5.0 Pharmacokinetic Time Course: Considerations for Labor & Delivery* describes the PK profile of chlorpyrifos and discusses the specific lifestage context of the CCCEH cohort for interpreting the biomarker data.

- *Section 6.0 Evaluation of CCCEH Cord Blood Data & Predicted Exposures to the Cohort* provides a characterization of the predicted chlorpyrifos exposures to women in the cohort, particularly during the period of indoor residential use prior to the voluntary cancellation of most residential uses in 2000. This characterization includes evaluation of potential exposures from food, water, and indoor residential exposure and uses the

PBPK model to predict internal doses of chlorpyrifos. The residential exposure characterization provides a range of potential exposures and concludes that the distributions of reported blood concentrations in the CCCEH cohort are consistent with the exposure profile of the time period of the epidemiology studies.

- *Section 7.0 Deriving a Point of Departure (PoD) for Neurodevelopmental Outcomes* of the document describes the conceptual approach to using the cord blood data to derive PoDs for women of childbearing age (13-49 years old), infants, and children. Section 7.0 also contains the proposed internal PoD for these lifestages.

- *Section 8.0 Assessing Extrapolation/Uncertainty* provides an evaluation of uncertainty in the context of the intra-species factor and the statutory FQPA 10X Safety Factor.

- *Section 9.0 Proposed Approach to Deriving Internal Dose Estimates: Integration of Exposure Assessment & PBPK Modeling* proposes an approach to use the PBPK model to estimate internal doses of chlorpyrifos from current food, water, and occupational exposures. Case study examples are provided for consideration.

- *Section 10.0 Discussion & Next Steps* describes how the analysis included in this issue paper addresses many of the uncertainties identified by the 2012 SAP regarding using the CCCEH data for deriving a PoD and describes next steps for the chlorpyrifos proposed tolerance revocation.

- *Section 11.0 and Appendices* provide citations from the document and supplemental information on relevant to the science issues in this issue paper.

- As part of the package of materials for the FIFRA SAP (but in separate files from this document), the agency has included all PBPK code needed to run the simulations described in the following sections and also associated output of the PBPK simulations.

3.0 Brief Summary of CCCEH Findings and Maternal & Cord Blood Levels

EPA has conducted systematic reviews of the scientific literature on epidemiology studies on neurodevelopmental outcomes associated with OP exposure in 2012, 2014, and 2015. Although other studies exist, the most robust epidemiology studies are conducted through three major U.S. based prospective birth cohort studies: 1) Mothers and Newborn Study of North Manhattan and South Bronx conducted by Columbia University, referred to in this document as "CCCEH;" 2) Mount Sinai Inner-City Toxicants, Child Growth and Development Study, or the "Mount Sinai Study/Cohort;" and 3) Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley, or "CHAMACOS Study/ Cohort." Investigators from each study cohort utilized a similarly strong study design (prospective birth cohort); measured pesticide exposure using several different methods including environmental indicators as well as specific and non-specific biomarkers of OPs;

ascertained developmental outcomes using validated assessment tools well-established in both clinical and research settings; and, measured, analyzed, selected and statistically adjusted for potentially confounding variables including socio-economic status and other environmental exposures using reasonable and appropriate methods.

The CCCEH study measured parent chlorpyrifos in cord blood, and other indicators (*e.g.*, air sampling, behavioral information), as etiologic measures of exposure, while the other two birth cohorts measured non-specific urinary metabolites of chlorpyrifos and other OPs (TCPy, dialkyl phosphate metabolites) in the mothers to estimate pesticide exposure. Therefore, EPA considers the CCCEH Mothers and Newborn Study research results as most relevant to the chlorpyrifos HHRA and is thus the focus on this analysis. The other two cohorts provide important supporting information.

The CCCEH Mothers and Newborn study participants were likely exposed to OPs through the diet and through residential use of the pesticide for indoor pest control. In the residential setting, study populations were most likely exposed through indoor residential use of the pesticide during the study time period and additionally exposed to OPs via the oral route through ingesting residues in the diet, from hand-to-mouth contact with in-home surfaces, as well as possible dermal or inhalation exposure through contact with treated areas in the home environment (Berkowitz *et al.*, 2003; Whyatt *et al.*, 2003; Whyatt *et al.*, 2009; Whyatt *et al.*, 2007). The time period under study within CCCEH study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, and researchers were able to show the change in exposure before (high use period) and after (low/no use period) the period of removal of chlorpyrifos products from the residential marketplace. EPA and the FIFRA SAP (2008, 2012) have concluded that "chlorpyrifos likely played a role in the neurodevelopmental outcomes observed in these [epidemiology] studies."

Researchers across the three children's health cohorts utilized the Bayley Scales of Infant Development II (BSID-II) to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) to assess neurodevelopment in early childhood. In the CCCEH Mothers and Newborn study, Rauh *et al.* (2006) investigated MDI and PDI at 12, 24, and 36 months of age. Children were categorized as having either high (>6.17pg/g) or low (≤6.17pg/g) prenatal chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics (Whyatt *et al.*, 2004). Authors reported that the difference in MDI scores was "marginally significant" (p=0.06) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed (Rauh *et al.*, 2006). Regarding the PDI score (motor skills), none of the 12 or 24 month PDI scores showed significant effects, but the 36 month score was significantly related to chlorpyrifos exposure. Motor skill deficits observed in Rauh *et al.* (2006) appear to continue in Rauh *et al.* (2015) with hand tremors while writing at approximately age eleven years.

Researchers noted that the effects were most pronounced at the 36 month testing period. Within the 36 month testing period, the likelihood of highly exposed children developing mental delays were significantly greater (MDI: 2.4 times greater (95% CI: 1.12-5.08, p=0.02) and PDI: 4.9 times greater (95% CI: 1.78-13.72; p=0.002)) than those with lower prenatal exposure (Rauh *et al.*, 2006). With respect to the findings related to the autism spectrum, from CCCEH, Rauh *et al.* (2006) reported a large odds ratio for pervasive developmental disorder (PDD) (OR=5.39; 95% CI: 1.21-24.11) when comparing high to low chlorpyrifos exposure groups. Regarding attention problems, Rauh *et al.* (2006) also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems (p=0.010), and in the more specific DSM-IV scale for ADHD problems (p=0.018).

To measure intelligence among school aged children, authors from each of the three children's health cohorts used the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV). The instrument measures four areas of mental functioning: the Verbal Comprehension Index, the Perceptual Reasoning Index, the Working Memory Index, and the Processing Speed Index. A Full-Scale IQ score combines the four composite indices. WISC-IV scores are standardized against U.S. population-based norms for English and Spanish-speaking children (Weschler, 2003). In the CCCEH Mothers and Newborn Study, Rauh *et al.* (2011) evaluated the relationship between prenatal chlorpyrifos exposure and neurodevelopment among 265 of the cohort participants who had reached the age of 7 years and had a complete set of data including prenatal maternal interview data, prenatal chlorpyrifos marker levels from maternal and/or cord blood samples at delivery, postnatal covariates, and neurodevelopmental outcome data (Rauh *et al.*, 2011). The linear regression reported by Rauh *et al.* (2011) for working memory is the source of information used by the agency in Section 7.0 for deriving a PoD for neurodevelopmental effects.

Figure 1 is supplemental information summarizing the distribution of blood concentrations in cord blood and maternal blood across time in the CCCEH cohort. This information was provided to the agency upon request of Columbia in 2015 to aid in this analysis. This figure provides important temporal information that helps characterize the blood levels of chlorpyrifos in years of high indoor use (1998/1999) compared with those after the voluntary cancellation (2001–2004) with substantial reduction in blood levels in 2001–2004 compared to 1998–2000. There is a notable difference in the magnitude of chlorpyrifos reported across these years. Prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only 16% of the measured values were greater than the limit of detection (LOD). In Figure 1, values listed as 0.2500 represent those below the LODs of 0.5 pg/g or 1.0 pg/g. Whyatt *et al.* (2003, 2009) have shown that levels of chlorpyrifos in maternal blood and umbilical cord blood levels were highly correlated ($r = 0.9$, $p<0.001$, $n = 64$; $r = 0.76$, $p<0.001$, n=180 mother-child pairs). Similarly, Figure 1 shows that cord blood and maternal blood levels of chlorpyrifos are generally similar across years of the study and across

the distribution. However, the levels of chlorpyrifos in maternal blood samples are slightly higher for 1998/1999 compared to cord blood.

Figure 1. Summary information on blood levels of chlorpyrifos provided by CCCEH to EPA (best copy available)

Cord and Maternal CPF (pg/g)
Percentiles, All Years Combined: 1998-2004

		CR_CHLOP CHLOROPYRIFOS RESULT CORD	MR_CHLOP CHLOROPYRIFOS RESULT MOTHER
N		424	427
Percentiles	5	2500	2500
	10	2500	2500
	25	2500	2500
	50	5850	5500
	75	3 8975	3 9000
	90	8 6500	7 8200
	95	12 0000	12 0000

Cord and Maternal CPF (pg/g))
Percentiles by Year: 1998-2004

YEAR_X			CR_CHLOP CHLOROPYRIFOS RESULT CORD	MR_CHLOP CHLOROPYRIFOS RESULT MOTHER
1999 (includes small # of 1998 values)	N		138	72
	Percentiles	5	2500	8250
		10	2500	1 5000
		25	2500	2 6000
		50	3 7750	6 7000
		75	8 8000	9 5300
		90	12 1000	16 0000
		95	15 0500	19 3500
2000	N		110	120
	Percentiles	5	2500	2500
		10	2500	2500
		25	1 6125	1 3325
		50	2 5200	3 8000
		75	4 3250	5 8000
		90	6 2900	9 1500
		95	8 8180	12 9500
2001	N		73	86
	Percentiles	5	2500	2500
		10	2500	2500
		25	2500	2500
		50	2500	2500
		75	5800	8675
		90	2 4460	2 4410
		95	2 5820	2 6125
2002	N		37	60
	Percentiles	5	2500	2500
		10	2500	2500
		25	2500	2500
		50	2500	2500
		75	9050	1 0950
		90	2 3700	2 2360
		95	2 5720	2 4975
2003 (includes small # of 2004 values)	N		68	89
	Percentiles	5	2500	2500
		10	2500	2500
		25	2500	2500
		50	2500	2500
		75	2500	2500
		90	2500	2500
		95	2500	2500

4.0 Lifestages for Consideration

For the 2014 HHRA, the agency developed PoDs based on AChE inhibition to protect against cholinergic toxicity; such cholinergic toxicity could occur to any lifestage if exposure is sufficiently high. As such, in 2014, the agency evaluated the spectrum of lifestages from the fetus through adulthood (Appendix 1). At this point in time, the focus of the assessment has transitioned to neurodevelopmental outcomes such that the critical lifestages of concern are fetuses, infants (< 1 year old), and children (1–2 years old) because EPA believes these outcomes may occur at exposure levels below those resulting in AChE inhibition. Fetuses may be exposed to chlorpyrifos through the mother while infants and children may be exposed directly. As discussed in Appendix 3, studies in laboratory animals do not suggest any specific critical period or lifestage but instead suggest pre- and post-natal periods of susceptibility. The agency acknowledges that the epidemiology literature regarding associations between post-natal (infancy, childhood) biomarker metrics and neurodevelopmental outcomes is limited to the Bouchard et al (2010) study, a cross-sectional study that observed positive association between attention and behavior problems and total DAPs and DMAPs, using urinary NHANES data in children 8–15 years old. The OP exposure being assessed in many of the epidemiology studies used concentrations of urinary dialkyl phosphate metabolites (DAPs) as the urinary biomarker. Total DAPs is a non-specific measure of OP exposure and is the sum of six separate molecules — three dimethyl alkylphosphate (DMAP) molecules of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate (DMP, DMTP, and DMDTP), and three diethyl alkylphosphate (DEAP) molecules of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate (DEP, DETP, and DEDTP) (for further details on DAPs, see Appendix A.3.1.3.2). The other studies which evaluated postnatal biomarker metrics and neurodevelopment outcomes have found no statistically significant associations. Specifically, postnatal exposure to OPs (measured as DAPs) has been assessed in the CHAMACOS cohort (Eskenazi *et al.*, 2007; Young *et al.*, 2005; Bouchard *et al.*, 2011) and two other cross-sectional studies (Guodong *et al.*, 2012; Oulhote and Bouchard, 2013). Despite the limited epidemiological evidence from postnatal exposure, the agency is proposing to use the chlorpyrifos cord blood data from CCCEH as the most relevant source of information for deriving a PoD specific for chlorpyrifos for fetuses, infants, and children.

The cord blood data are directly relevant to fetal exposure; however, they may be less relevant to older children. As described later in this document, drinking water risk was one of the areas identified in the 2014 HHRA as having a high exposure potential. Early infant [exposure through feeding via formula in bottles (made with water)] is highlighted in the case studies in Section 9.0. Because this group consumes formula reconstituted with water frequently and throughout the day and night, combined with lower body weight and lower metabolic capacity, this may lead to relatively high internal exposure to chlorpyrifos. The agency has not included any case study evaluations for children 1–2 years old, a group which often provide the highest

food exposure. Children 1–2 years old are not included as these ages are temporally removed from gestational exposure; as such the relevance of such data to predict the outcomes in toddlers is unclear.

The current model being used by the agency for the chlorpyrifos risk assessment does not include gestation or lactational exposure. With respect to lactational exposure, the agency is aware of breast milk monitoring data from the Pilot study for the National Children's Study (NCS) Supplemental Methodological Studies (SMS)[2] that can be used to evaluate infant exposure to chlorpyrifos through breastfeeding. These data have not been used in this issue paper as a case study as the breastmilk case study would be similar to the drinking water, bottle feeding infant scenario included in Section 9.3.4.2 and thus not provide any additional scientific understanding for developing the risk assessment approach for using the cord blood. Moreover, the agency does have information on the participants in the breast milk study to evaluate how different sources of exposure impact breast milk levels. The agency may consider, if appropriate, the breast milk monitoring data as part of the tolerance revocation. Since there are some (albeit limited) breast milk monitoring data from contemporary exposures, the lack of a lactational PBPK model does not introduce substantial uncertainty in the risk assessment.

In April 2015, DAS modified the multi-route PBPK/PD model for chlorpyrifos to include additional code to describe physiological changes for women during pregnancy (Poet, 2015). The gestational component of the model includes the following modifications: (1) placenta, uterine, and fetal compartments, all of which grow over the course of pregnancy; (2) pregnancy-specific changes in the fat, rapidly-perfused, and slowly-perfused compartments; (3) pregnancy-specific changes in blood composition resulting in increased blood volume and decreased hematocrit; and (4) pregnancy-specific changes in metabolism, both CYP450s and PON1 enzymes based on published studies. No changes were made to the PD model since there are no data available to suggest cholinesterase binding changes during pregnancy. The growth of fetus, uterus, and placenta predicted by the model agreed with empirical data as were the changes in tissue and blood volume (Abduljalil, *et al.*, 2012). While the modified model reasonably simulated the physiological changes during pregnancy, the model's predictive ability to simulate internal dosimetry of chlorpyrifos cannot be properly evaluated since there are no chlorpyrifos-specific pharmacokinetic data available during pregnancy. *As such, the agency cannot evaluate its predictive capacity and thus, the pregnancy model will not be used for risk assessment at this time.*

The agency does note, however, that the pregnancy model was built based on the best knowledge available and some preliminary simulations using this model suggested that when accounting for variability in physiology, pregnant women are not more at risk than non-pregnant women. Given the same oral dose, blood concentrations of chlorpyrifos in women in the third trimester were

[2] https://www.nichd.nih.gov/research/ncs/Pages/default.aspx

slightly lower than those in non-pregnant women (Appendix 6). While some parameter changes during pregnancy contributed to more chlorpyrifos in blood (*e.g.*, smaller fat:blood partition during pregnancy) and other changes contributed to less chlorpyrifos in blood (e.g., increased tissue volumes for more storage), the combined effects from these multiple physiological changes during pregnancy resulted in slightly lower blood concentrations of chlorpyrifos during pregnancy. Similarly, RBC AChE inhibition in pregnant women at a given dose was comparable to non-pregnant women.

In addition, data from CCCEH suggest that maternal blood is a reasonable surrogate for cord blood. Figure 1 provides cord blood and maternal blood values across the CCCEH cohort and there is strong agreement across the years on study and across the distribution. Whyatt *et al.* (2009) have shown that levels of chlorpyrifos in maternal blood and umbilical cord blood levels were highly correlated ($r = 0.9$, $p<0.001$, $n = 64$). In a second paper, Whyatt *et al.* (2003) also showed that chlorpyrifos in maternal blood and umbilical cord blood levels were highly correlated ($r = 0.76$, $p<0.001$, $n=180$ mother-child pairs). These together suggesting that tracking the blood concentrations of the mother is a reasonable surrogate for the fetus. Although the agency would prefer to have a robust gestational PBPK model parameterized with chlorpyrifos data, when considered together—based on the preliminary results of the pregnancy PBPK model and strong correlation between maternal and cord blood in the CCCEH cohort (Figure 1) the lack of such model robust does not add major uncertainty into the 2016 analysis. *The agency can reliably estimate blood levels of chlorpyrifos for females of childbearing age.*

5.0 Pharmacokinetic Time Course: Considerations for Labor & Delivery

An example of time course profile for chlorpyrifos in blood is shown in Figures 2a and 2b for current occupational exposures to pesticide handlers. The agency uses the term handlers to describe those individuals who are involved in the pesticide application process. Similar figures for food, water, and residential exposures are shown throughout the document in subsequent sections. As shown in Figures 2a and 2b, the time course profile shows a consistent pattern of a daily, rapid increase in internal dose during the exposure period followed by a rapid decline after the exposure period ends. The rapid decline of chlorpyrifos after exposure terminates is expected given the rapid metabolism of chlorpyrifos. The rapid increase periods represent rapid uptake during activities that lead to chlorpyrifos exposures, while the rapid decrease periods are primarily attributed to distribution from the central compartment (circulation) into the peripheral compartments (body tissues), loss to metabolism, and binding to esterase. For chlorpyrifos, the half-life of this initial phase is estimated to be approximately four hours.

Figure 2b shows that after the exposure ends the internal dose curve flattens as sequestered chlorpyrifos slowly becomes available for clearance. During this terminal clearance phase, the half-life is a function of both clearance (i.e., metabolism and excretion) and distribution of chemical between tissues and blood; the chemical is eliminated only when it is distributed to the

clearing organs (e.g., liver for metabolism, kidneys for excretion) (Toutain, and Bousquet-Melou, 2004). Depending on the size of the peak, measuring plasma concentrations during the terminal elimination phase can be challenging because these levels may fall below the detection/quantification limits of the analytical techniques. Thus, to estimate the plasma terminal half-life for chlorpyrifos, the PBPK model was used to predict the time course of chlorpyrifos concentrations in plasma during the exposure period and 30 days after exposure. Two scenarios were simulated to ensure that the model estimated the same terminal half-life for different routes of exposure. The two scenarios were: (a) a five-day, 8 hours/day, dermal and inhalation exposure; and (b) a daily single oral exposure for 5 days, and the estimated terminal half-life from both scenarios is approximately 120 hours (i.e., 5 days).

Figures 2a and 2b. PBPK model-predicted time course of chlorpyrifos in blood after 8 hours/day, 5 days/week exposures for two weeks (2a: full profile; 2b: sub-set of Figure 2a)

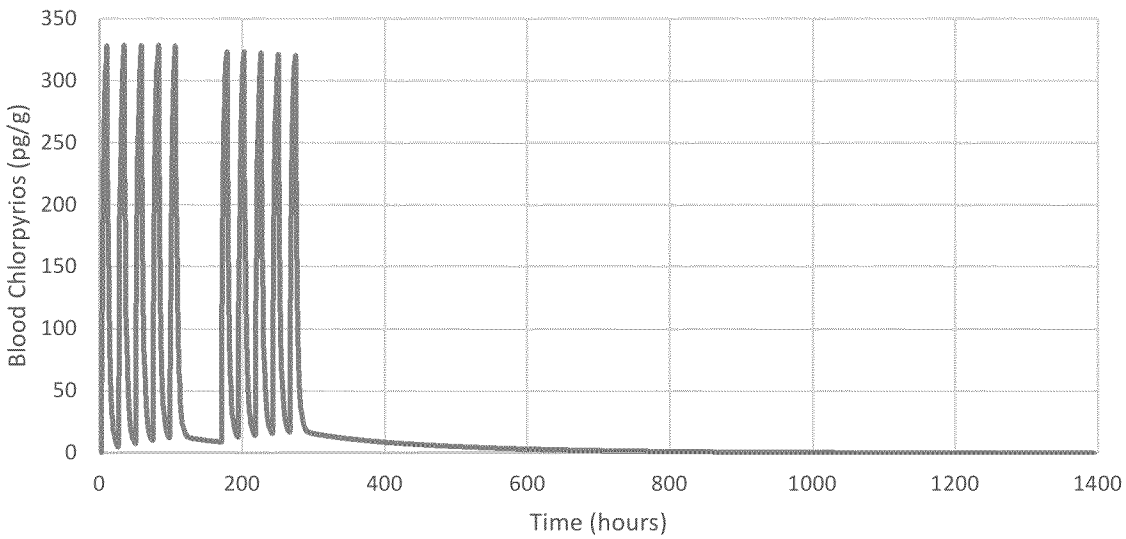


Figure 2a. PBPK model-predicted time course (full profile) of chlorpyrifos concentrations in venous blood (pg/g) resulting from the occupational handler exposure scenario, mixing/loading liquids (EC) for cole crops at 1 lb ai/A.

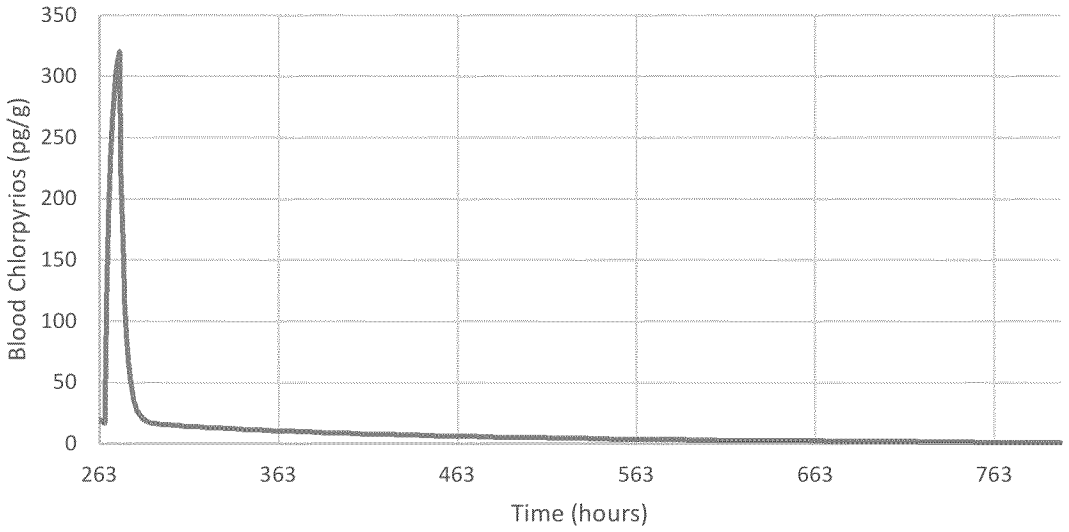


Figure 2b. PBPK model-predicted time course (sub-set of the above Figure 2a) of chlorpyrifos concentrations in venous blood (pg/g) after the last day of exposure resulting from the occupational handler exposure scenario, mixing/loading liquids (EC) for cole crops at 1 lb ai/A.

For interpreting the CCCEH blood levels of chlorpyrifos (Figure 1), it is important to consider these PK time course patterns in the context of pregnant women going into labor and ultimately delivering a newborn in the hospital. Whyatt *et al.* (2003) note that a sample of umbilical cord blood was collected as close to delivery as possible and a sample of maternal blood was obtained within 2 days postpartum. Reported times for labor and delivery in a first pregnancy range from 8-20 hours and may become faster for subsequent pregnancies (www.webmd.com; www.mayoclinic.org; www.parents.com; www.babycenter.com). Some of this time period would have been spent in labor at home while the remainder would have been at the hospital. The time spent at home vs. the hospital are not known and are likely to have varied among the women in the cohort. In a systematic review of 18 reported mean 'active labor' duration studies, Neal et al (2010) note that in contemporary medicine, women are most often admitted to the hospital labor unit when cervical dilation is expected to accelerate. The onset of the active phase generally begins at dilation between 3 cm and 5 cm and in the presence of regular uterine contractions. Neal *et al.* (2010) report than among healthy, low-risk, nulliparous women at term with a spontaneous labor onset, the 'active phase' of labor lasted an average of 6.0 hours, and up to 13.4 hours at two standard deviations from the mean.

Most births in the US are vaginal. The agency is aware that in the US Cesarean section rates have increased from approximately 20% in 1996 to 33% in 2011 (ACOG, 2014). CCCEH investigators have not reported the rates of vaginal vs. Caesarean deliveries in the cohort. Some Cesarean sections are performed after labor has started for medical reasons associated with the safety of the baby or the mother (and thus would be covered by the above description). However, in the context of this document and interpreting the chlorpyrifos biomonitoring data, it

would be the *planned* Cesarean sections, where the mother enters the hospital and within a short period of time after arrival delivers her baby, which could potentially impact the current evaluation. Although planned Cesarean sections have similarly risen in recent years, reported rates are not high, ranging from 4–18% of pregnancies (Roberts *et al.*, 2015; David *et al.*, 2015; Black *et al.*, 2015). Given the relatively low frequency of planned Cesarean sections and the consistency of the agency's exposure characterization with the CCCEH cord blood and maternal blood biomonitoring data, the impact of short delivery times from *planned* Cesarean sections does not contribute significant uncertainty in the agency's evaluation or proposed approach.

As shown in Figure 2b, upon ending an exposure period, there is a rapid decline in internal dose; this rapid decline is driven by the initial half-life of 4 hours and lasts for six to ten hours. The time of rapid decline (6–10 hours) is consistent with the average time of active labor in the hospital reported by Neal *et al.* (2010). In other words, at six to ten hours after the exposure ends, the internal dose profile is at the low point on Figure 2b. In the context of the CCCEH cohort, the end of chlorpyrifos exposure represents leaving the apartment and delivering a baby at the hospital. As such, there is a reasonable likelihood that the reported cord blood levels in the CCCEH publications do not represent the peak levels or levels in the rapid decline phase. Instead, given a labor and delivery scenario, it is more likely that the CCCEH blood samples were collected at or near the low points in the PK profile, especially for those individuals whose babies were delivered ten or more hours after chlorpyrifos exposure. Further, given that in the CCCEH cohort, maternal blood samples were collected "within 2 days postpartum" and not "as close to delivery as possible" like the cord blood, it is likely that the maternal blood samples were collected during the terminal clearance phase where the half-life is 120 hours and when the blood levels were not changing rapidly. For example, in the terminal half-life phase of the PK profile, it takes two days for blood concentration of chlorpyrifos to only drop from 8.8 pg/g to 6.7 pg/g; or it takes one day to drop from 7.7 pg/g to 6.7 pg/g.

In summary, during periods of exposure and immediately after exposure, blood concentrations rapidly increase and decrease which results in a daily sawtooth pattern. Upon cessation of the exposure, the terminal half-life (approximately 120 hours) predominates resulting in an asymptotic appearance for the internal dosimetry. During this terminal half-life phase, the internal doses are changing slowly.

For deriving the proposed PoDs (below), the agency is assuming the CCCEH levels do not represent values with the rapid increase/decrease phase. Instead, the agency is assuming the reported values for cord blood and maternal blood are at or near the low points or likely within the terminal clearance period (and thus unlikely to change significantly across several days). As such, the agency is reporting two values in the scenarios in Sections 6.0 and 9.0 below: a) 10 hours post-peak exposure, and b) 24 hours post-peak exposure.

6.0 Evaluation of CCCEH Cord Blood Data & Predicted Exposures to the Cohort

As an initial step towards determining a PoD based on the neurodevelopmental outcomes in the epidemiology studies, the agency has developed an analysis:

1) to characterize the source and magnitude of chlorpyrifos exposure to the women in the CCCEH cohort by considering drinking water, food, and residential exposure potential; and
2) to compare PBPK model predicted blood levels across a range of exposure scenarios to blood levels reported by CCCEH.

The PBPK model has been used to predict blood levels in women across exposure scenarios for comparison with the cord blood levels reported by the CCCEH. Drinking water exposure (Section 6.1), food exposure (Section 6.2), and residential exposure (Section 6.3) are described below.

6.1 Drinking Water Exposure to the CCCEH Cohort

New York City has a watershed protection plan[3] in order to avoid filtration requirements as part of the drinking water treatment process. While this watershed protection plan contains objectives to prevent pollution from non-agricultural and agricultural operations through a voluntary program (*e.g.*, best management practices established by the USDA Natural Resources Conservation Service), the program does not specifically target a reduction or elimination of surface water contamination from pesticides.[4] This protection plan may not completely prevent chlorpyrifos or chlorpyrifos-oxon from reaching surface water used to supply drinking water to New York City (specifically in the areas where participants of the CCCEH study lived: Harlem, Washington Heights and South Bronx). OPP has evaluated the likelihood that chlorpyrifos or its oxon may have been present in the drinking water of mothers in the CCCEH cohort and has concluded exposure to chlorpyrifos and chlorpyrifos-oxon via drinking water was unlikely.

Approximately 99 percent of New York City's drinking water was sourced from three upstate watersheds (*i.e.*, impounded in the Croton and Catskill-Delaware Systems) during the CCCEH study period. The watersheds are shown in Figure 3. Figure 4 shows an overlay of surface water intakes, corresponding watersheds and agricultural land.

[3] http://www.dos.ny.gov/watershed/nycmoa.html
[4] http://www.nycwatershed.org/agriculture/planning/

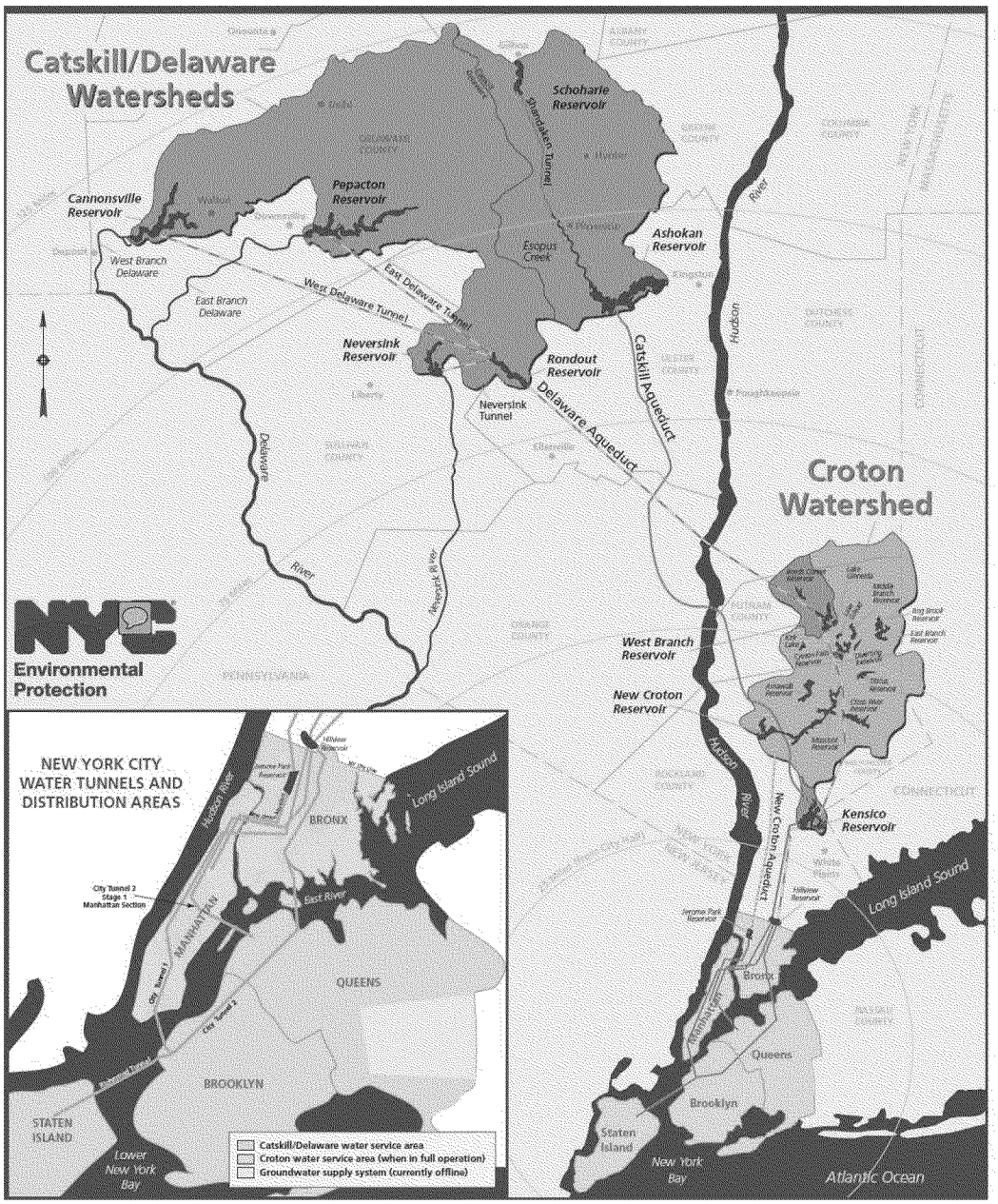


Figure 3. New York City's Water Supply System[5]

[5] New York City Environmental Protection, New York City 2013 Drinking Water Supply and Quality Reporthttp://www.nyc.gov/html/dep/pdf/wsstate13.pdf

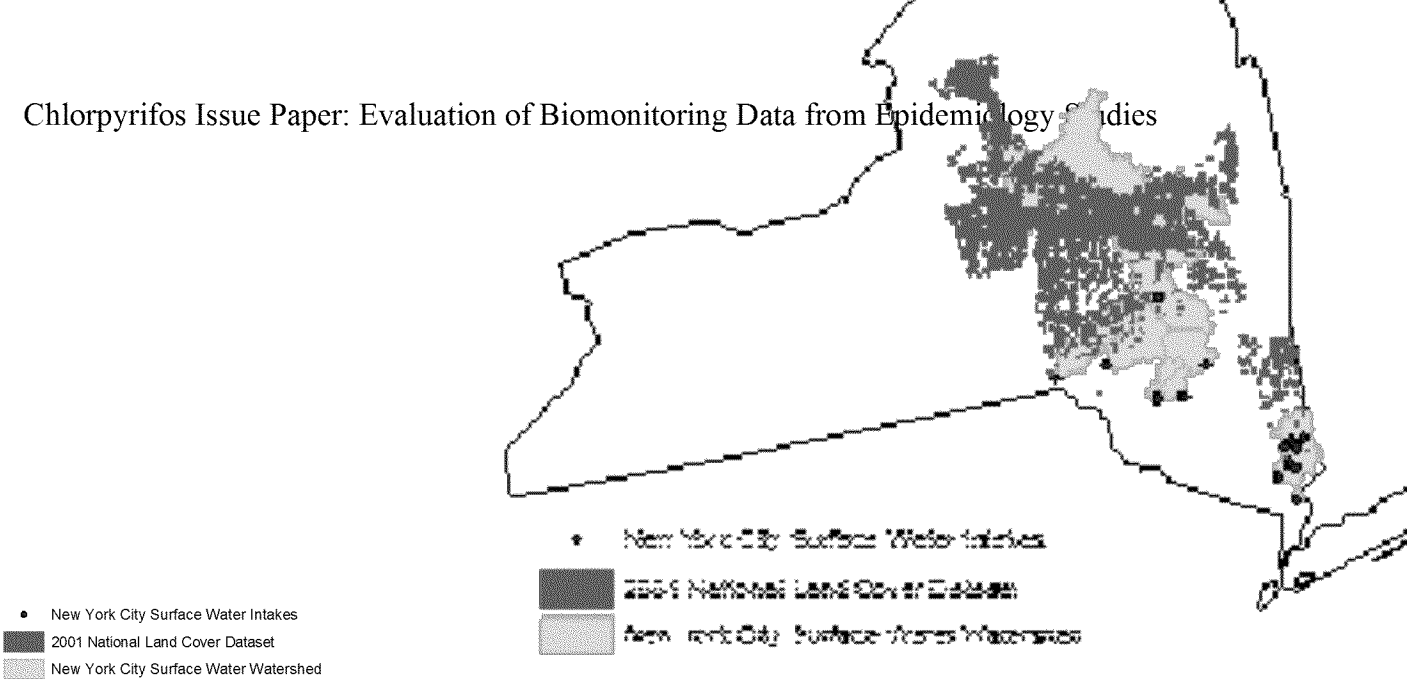


Figure 4. New York City Surface Water Intakes, Corresponding Watersheds and Agricultural Cropland Overlay[6]

Many of the intakes are located in areas where large scale chlorpyrifos use (*i.e.*, agricultural) is not expected, suggesting exposure would have been low. The overlap of potential non-agricultural chlorpyrifos use sites is unknown. However, the highest overlap of potential agricultural chlorpyrifos use sites with the intake watersheds suppling the Catskill-Delaware System is approximately 30 percent (Figure 3).

Examination of the USGS National Water-Quality Assessment Program (NAWQA)[7], USEPA/USGS Pilot Reservoir Monitoring Program[8], and USDA Pesticide Data Program (PDP)[9] indicates a very low detection frequency of chlorpyrifos and no detections of chlorpyrifos-oxon for sample sites located within New York State. The low detection frequency may be an artifact of the program design such as not being targeted to chlorpyrifos application or not sampling frequently enough. Another explanation for the low detection frequency is that the watershed protection plan is effective at limiting chlorpyrifos in New York City drinking water sources. EPA's analysis of the monitoring data for the years 1997–2004 (Table 1) suggests the highest potential concentration of chlorpyrifos during the CCCEH study period was 0.25 µg/L in 2003 and 2004 for the watersheds used as source drinking water for New York City. This exposure concentration is based on one half of the reported limit of detection. The New York City drinking water supply and quality reports[10] report no measured concentrations of chlorpyrifos[11]; however, the detection level and the number of samples collected by the community water

[6] Overlay developed by EPA (R. Bohaty).
[7] http://water.usgs.gov/nawqa/
[8] http://archive.epa.gov/pesticides/carat/web/pdf/dw5.pdf
[9] https://www.ams.usda.gov/datasets/pdp/pdp-drinking-water-project
[10] http://www.nyc.gov/html/dep/html/home/home.shtml
[11] Chlorpyrifos sampling began in 2002

system is not reported. Mixing of sourced water as well as drinking water treatment (described below) are expected to reduce the potential drinking water exposure to chlorpyrifos.

Table 1. Estimated Exposure Based on Evaluation of Ambient Monitoring Data for New York City Community Water System Watersheds

Year	1997	1998	1999	2000	2001	2002	2003	2004
Concentration (µg/L)	0.002	0.015	0.015	0.06	0.0423	0.0025	0.25	0.25
Bold font indicates measured concentrations. Other referenced concentrations are ½ the limit of detection. Shading indicates highest potential exposure.								

The Catskill-Delaware System supplies approximately 90 percent of the surface sourced drinking water to New York City. Prior to 2013, the Catskill-Delaware drinking water treatment facility only used chlorine to disinfect water entering the drinking water distribution system. As such, any chlorpyrifos present is expected to have oxidized to chlorpyrifos-oxon during drinking water treatment and, therefore, chlorpyrifos would not have been present in drinking water and by extension would not have been measured in cord blood from this exposure route. In addition to chlorine, sodium hydroxide was added to Catskill-Delaware water to increase pH and reduce corrosivity.[12] Examination of the New York City Drinking Water Supply and Quality Reports for years suggests the pH of distributed drinking water ranges from a minimum of 6.7–6.9 to a maximum of 8.3–9.8. The pH ranged from 6.0–8.4 during the CCCEH study period. Both chlorpyrifos and chlorpyrifos-oxon are more rapidly hydrolyzed under basic (pH>7) conditions. Mixing of sourced water from other intakes is also expected to reduce potential exposure to chlorpyrifos-oxon as a result of oxidation of chlorpyrifos.

In summary, exposure to chlorpyrifos is not expected to have occurred via New York City drinking water. Therefore, the chlorpyrifos measured in cord blood for the CCCEH cohort would not have been the result of exposure to chlorpyrifos via drinking water. Exposure to chlorpyrifos-oxon in drinking water was also not likely, with any exposure being very low.

6.2 Food Exposure to the CCCEH Cohort

The agency uses probabilistic exposure approaches for assessing food exposure that have had extensive peer review through the FIFRA SAP (2000a; 2000b) and public comment; these approaches and associated sources of residue data can be found in Section 9.2 below. The agency expects that exposure to chlorpyrifos (but not the oxon) in food may have occurred to the women in the cohort over the entire period of the CCCEH chlorpyrifos publications. In 2000, EPA required that all uses of chlorpyrifos products in the U.S. be discontinued on tomatoes. Use on apples was restricted to pre-bloom and dormant application. The grape tolerance was lowered to reflect the labeled dormant application. In 2002, EPA limited the use of chlorpyrifos on citrus

[12] New York City Environmental Protection http://www.nyc.gov/html/dep/pdf/wsstat00b.pdf

and tree nuts as well other crops. However, chlorpyrifos use on numerous crops remained after this mitigation.

The agency performed simulations of food exposure across the distribution of food exposures from the 2014 HHRA. In the food exposure simulations, females of childbearing age were exposed once daily for 30 consecutive days. For example, Figure 5 shows the predicted blood profile of a women of childbearing age from once daily food exposure at the 99.9th percentile from the 2014 HHRA calculated using the PBPK model following 30 consecutive days of exposure. As shown in the graph after 30 days of exposure, the peak blood level is 7.14 pg/g, a value near the lower limit of the top tertile reported by CCCEH (6.17 pg/g). However, the blood levels drop quickly each day after the end of exposure. By eight hours post-exposure, the blood levels have dropped to 3.2 pg/g; and between 13–16.5 hours post-exposure, the blood levels are below the LOD.

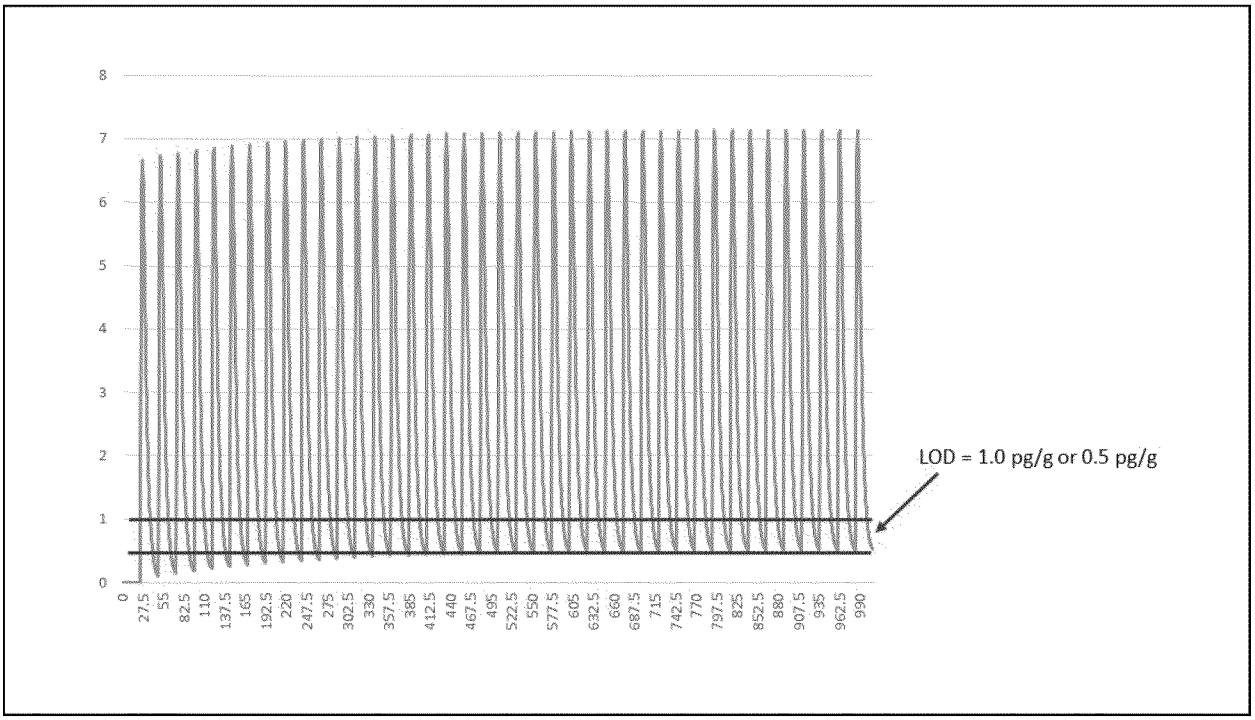


Figure 5. Time course of venous blood for female of childbearing age consuming 99.9th percentile of food for 30 consecutive days.

As shown in Table 2, from the 10–70th percentile, the peak values do not go over the LOD of 0.5 and/or 1.0 pg/g and at the 90th percentile, the blood levels are only above the LOD for four hours.

Table 2. Predicted levels of food exposure to chlorpyrifos from the 2014 revised risk assessment (Drew, 2014) and associated maximum and 24 hour blood concentrations in adult females

Percentile of Exposure	Exposure from Calendex	Max Blood Levels of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex	10-hour Blood Levels Post Exposure Values of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex	24-hour Blood Levels Post Exposure Values of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex
10	0.003 µg/kg/day	0.29	0.060	0.021
30	0.005 µg/kg/day	0.48	0.099	0.034
50	0.007 µg/kg/day	0.67	0.139	0.048
70	0.009 µg/kg/day	0.86	0.179	0.062
90	0.014 µg/kg/day	1.33	0.278	0.096
95	0.018 µg/kg/day	1.71	0.358	0.124
97.5	0.023 µg/kg/day	2.19	0.457	0.158
99	0.029 µg/kg/day	2.76	0.576	0.200
99.5	0.037 µg/kg/day	3.52	0.735	0.255
99.9	0.075 µg/kg/day	7.14	1.490	0.517

The exposure period after the voluntary cancellation of indoor use would have included only dietary exposure. The agency's predicted blood concentrations from food exposure in Table 2 are consistent to the pattern of reported values in the CCCEH study with respect to the number of samples above the LOD before and after the voluntary cancellation of the indoor uses. As noted above, the CCCEH study enrollment time period (and collection of cord blood) spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment but extensive agricultural use remained. Prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only 16% of the measured values were greater than the LOD.

Figure 1 shows that from 2000–2002, there is an overall decrease in the reported levels of chlorpyrifos. By 2003/2004, none of the cord blood or maternal blood samples, at least up to the 95th percentile were above the LOD (Figure 1). Moreover, there was only one child born in the time period subsequent to the voluntary cancellation of chlorpyrifos in the residential marketplace for whom the cord blood chlorpyrifos level was in the upper-tertile of pre-cancellation exposure levels. Given the results in Table 2 and the results reported by CCCEH after the voluntary cancellation (Figure 1), it is unlikely that food exposure alone leads to high levels of *measured* chlorpyrifos in blood.

It is notable that CCCEH study is underpowered to detect adverse neurodevelopmental effects at blood concentrations substantially lower than those reported in Rauh *et al.*, (2006, 2011). Although the CCCEH investigators report before/after cancellation findings with respect to lack of adverse findings after the cancellation of indoor uses, it is important not to over interpret these findings to equate that remaining food exposure would not be associated with neurodevelopmental outcomes. Instead, there is uncertainty with respect to safe levels of remaining exposures.

6.3 Residential Exposure to the CCCEH Cohort

Based on the above evaluations for drinking water and food exposure, the agency believes the measured values of chlorpyrifos in the CCCEH come primarily from exposure in the home environment, particularly at the upper-tertile. It is important to note that the CCCEH investigators did not collect information on chlorpyrifos application timing; instead, the investigators have reported generic pesticide exposure information. Whyatt *et al.* (2002) reported that pest control measures were used by 85% of respondents, whether by housing superintendent, pest control operator (PCO), or by themselves. The majority reported regular use of a pesticide product, at least once per month. At the time of the initial recruitment period of pregnant mothers in the CCCEH studies (1997–1999), chlorpyrifos was being used widely for in-home pest control treatment. There were two risk mitigation actions which impact the interpretation of the CCCEH cord blood data (Figure 1):

- In January 1997, the technical registrants entered into an agreement with the agency to reduce indoor exposure to chlorpyrifos, especially to children and other sensitive groups. All residential broadcast and total release aerosol/foggers uses were cancelled — indoor crack and crevice (perimeter) and spot treatment as a termiticide were not included. The following chlorpyrifos uses were also cancelled: all direct application pet products including sprays, shampoos, and dips (pet collars not included); and all insecticidal paint additives. Further, all concentrates which required mixing were eliminated limiting the household consumer to ready-to-use products. Although the above uses were cancelled in 1997, existing stocks could be phased out, or applied until depleted.

- In June 2000, the technical registrants, entered into an agreement with the agency to eliminate or phase out nearly all remaining uses that resulted in residential exposure including home lawn, crack and crevice and other indoor uses. The only exceptions are

> baits containerized in child resistant packaging and public health uses such as mosquito and fire ant control. The agreement focused first on mitigation that achieved the greatest reduction for children. As such, any non-residential uses where children could be exposed, such as schools and parks, were also cancelled. For these uses, retailers had a stop sale date of December 31, 2001. A phase out of existing stocks was also allowed following the 2001 stop sale.

Given the lack of specific CCCEH exposure information, the agency developed a range of possible residential exposure scenarios resulting from residential post-application exposures to chlorpyrifos products available prior to the voluntary cancellation of indoor products in 2000. These possible residential exposure estimates were input into the PBPK model to predict a range of possible blood levels of chlorpyrifos in women.

A conceptually similar analysis was undertaken by the agency following the recommendation of the FIFRA SAP (2012) to conduct a "dose reconstruction" analysis of indoor residential uses to assess potential for RBC AChE inhibition. The dose reconstruction analysis was conducted and presented in the 2014 HHRA. The dose reconstruction analysis, including all methods and inputs, is described in Appendix 2 of this document. The goal of the dose reconstruction exercise was to estimate upper limit, bounding level exposures to test the hypothesis whether RBC AChE at or above the 10% inhibition level used by the agency for typical AChE PoDs may have occurred in the cohort. For example, in the dose reconstruction analysis, exposure to the women was assumed to occur 24 hours a day without adjustments for bathing, showering, or leaving the residence for 14 consecutive days. In contrast, the goal of the present 2016 analysis is to predict typical product usage and behaviors thereby deriving more accurate and realistic estimates of exposure.

The present analysis includes two parts: 1) development of six scenarios representing a range of possible exposure levels to women in the CCCEH cohort and 2) PBPK simulations using these exposure scenarios for prediction of internal dosimetry across time. These simulations are intended to mimic indoor application of chlorpyrifos with daily dissipation across a one month time period. The one-month time period was selected as the likely period between applications of chlorpyrifos. Since exposure information on frequency and magnitude of applications were not collected by CCCEH investigators, there is uncertainty in the degree to which the monthly application period is accurate. Similarly, in the CCCEH cohort, there is uncertainty in the timing of chlorpyrifos indoor application in relation to the timing for labor and delivery (*i.e.*, when the cord blood was collected).

6.3.1 Methods

For the current evaluation, the agency has focused on chlorpyrifos blood levels resulting from post-application exposures only. Whyatt *et al.* (2002) reported that many women applied pesticide themselves and that majority who reported using a pesticide used them at least once per month. However, as the agency has shown in the dose reconstruction (Appendix 2) analysis,

post-application exposures are greater in magnitude than the exposure which occurs during an application. As such, by focusing on post-application exposure, the agency is including the higher exposure potential. A total of six residential post-application exposure scenarios and corresponding PBPK model simulations have been assessed. Two exposure scenarios mimic potential indoor chlorpyrifos use patterns likely to have occurred amongst the CCCEH cohort using EPA standard exposure assessment approaches; these two scenarios represent the high end exposure potential. To estimate lower exposures, four additional PBPK model simulations were conducted with use of reported values from the CCCEH investigators. The six post-application exposure scenarios allow for establishment of ranges of potential exposures and comparison of predicted internal dosimetry levels to those reported by the CCCEH investigators.

All six scenarios share some exposure assumptions. For the present evaluation, the agency has assumed a once daily shower occurred immediately following exposure activities. All PBPK simulations conducted were run for 30 days post-application to be consistent with the monthly usage reported by CCCEH investigators. Daily exposure times for post-application adult dermal contact with carpets and hard surfaces were used as recommended in the 2012 Standard Operating Procedures for Residential Pesticide Exposure Assessment[13] (herein referred to as the 2012 Residential SOPs); specifically, how long an adult is expected to spend daily on a treated carpeted or hard floor. For adults, the recommended exposure times for post-application dermal exposure assessments are 8 and 2 hours daily for carpets and hard surfaces, respectively. These values are based on the EPA Exposure Factors Handbook 2011[14] Edition that provides information on the total time spent in a residence and time spent in various rooms within a residence. Additionally, chlorpyrifos residues were assumed to dissipate 10% daily; that is, the total amount of residue available for transfer from the treated floor is assumed to reduce by 10% for each subsequent day of exposure.

Two residential exposure scenarios (broadcast hard surface, perimeter carpet) have been evaluated using the same algorithms as those described in Appendix 2 for the dose reconstruction analysis for estimation of daily dermal and inhalation (inhalation exposures are limited to 2 hours on the day of application) chlorpyrifos post-application exposures. Consistent with the dose reconstruction analysis, the present analysis is based on the 2012 Residential SOPs methods which describe specific algorithms and inputs, on a scenario-specific basis; the 2012 Residential SOPs were subjected to peer review by FIFRA SAP in October 2009.[15] Specifically, the 2012 Residential SOPs have been used to predict the range of potential exposures which could have occurred to individuals in the cohort for broadcast hard surface and perimeter carpet treatments. Like the dose reconstruction analysis, the present analysis uses chemical-specific data inputs recommended in the 2012 Residential SOPS (*i.e.*, the fraction of chlorpyrifos residues transferred from treated carpet and hard surfaces to the exposed individual). Similarly, both the

[13] http://www.epa.gov/pesticides/science/residential-exposure-sop.html
[14] http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252
[15] http://www.regulations.gov/#!docketBrowser;rpp=50;po=0;D=EPA-HQ-OPP-2009-0516

dose reconstruction and 2016 typical exposure analysis uses registrant submitted chemical-specific exposures studies and exposure studies from the open literature relevant to the product formulations being assessed. In contrast, the present analysis uses PBPK model inputs more representative of typical product usage and residential behaviors than those used for the dose reconstruction analysis.

Unlike the two residential exposure scenarios described above, four additional exposure scenarios were evaluated without use of standard agency methods (*i.e.*, the 2012 Residential SOPs or chemical-specific exposure data). Instead, the highest reported blood chlorpyrifos value by Rauh, *et al.* (2011), 63 pg/g, was used as an anchor for evaluation of internal dosimetry following indoor chlorpyrifos exposures to women in the cohort. Two residential exposure scenarios were evaluated using the PBPK model to estimate, for a one month time period, what daily exposures would be required to result in a peak concentration of approximately 60 pg/g blood chlorpyrifos on the 30th (last) day modeled assuming both indoor broadcast hard surface (2 hour daily exposure) and perimeter carpet treatment (8 hour daily exposure). Two additional residential exposure scenarios were evaluated using the PBPK model to estimate, for a one month period, what daily exposures would occur following peak concentration of approximately 60 pg/g blood chlorpyrifos on the 1st (initial) day modeled assuming both indoor broadcast hard surface and perimeter carpet treatment.

The following six exposure scenarios were analyzed under the exposure and modeling conditions as described:

1. ***Broadcast hard surface:*** A broadcast treatment is the application of a pesticide product to the entirety of the hard surface area of the use site. This exposure scenario represents the highest of the indoor residential exposures for the 2016 evaluation assessed since it results in the most conservative daily exposure estimates and corresponding PBPK model outputs of blood chlorpyrifos. Although these uses were cancelled in 1997, existing stocks could be applied until depleted. Therefore, some exposure to the broadcast formulation was possible for the cohort. A daily exposure time of 2 hours/day was used as reported in the 2012 Residential SOPs for the duration an adult spends on a treated hard surface. A once daily shower, 10% daily residue dissipation, and a 30 day post-application period were assumed.

2. ***Perimeter, carpet:*** Given the cancellation of the broadcast uses in 1997, the perimeter treatment was likely the predominant exposure scenario for the cohort from 1998–2001. A perimeter treatment is a coarse application of a liquid pesticide in a wide band or strip of the use site, in this case, the perimeter of the carpeted room. A daily exposure time of 8 hours/day was used as reported in the 2012 Residential SOPs for the duration an adult spends on a treated carpet. Further, a once daily shower, 10% daily residue dissipation, and a 30 day post-application period were assumed.

3. ***Approximately 60 pg/g blood level on the 30th (last) day of the simulation using 8-hour exposure duration (for carpet exposure).*** The highest reported blood chlorpyrifos value by Rauh, *et al.* (2011) is 63 pg/g. Using approximately 60 pg/g as the peak on the last

day of simulation, the agency back-calculated the peak at the time of application assuming 10% dissipation rate per day, once daily shower, and 8 hours exposure per day.

4. ***Approximately 60 pg/g blood level on the 30th (last) day of the simulation using 2-hour exposure duration (for hard surface exposure).*** The highest reported blood chlorpyrifos value reported value by Rauh, *et al.* (2011) is 63 pg/g. Using approximately 60 pg/g as the peak on the last day of simulation, the agency back-calculated the peak at the time of application assuming 10% dissipation rate per day, once daily shower, and 2 hours exposure per day.

5. ***Approximately 60 pg/g blood level on day of chlorpyrifos application (day 1 of the simulation) using 8-hour exposure duration (for carpet exposure).*** The highest reported blood chlorpyrifos value by Rauh, *et al.* (2011) is 63 pg/g. Using approximately 60 pg/g as the peak on day of application (day 1 of the simulation). Following this peak value, a 10% dissipation rate per day, once daily shower, and 8 hours exposure per day was assumed.

6. ***Approximately 60 pg/g blood level on day of chlorpyrifos application (day 1 of the simulation) using 2-hour exposure duration (for hard surface exposure).*** The highest reported blood chlorpyrifos value by Rauh, *et al.* (2011) is 63 pg/g. Using approximately 60 pg/g as the peak on day of application (day 1 of the simulation). Following this peak value, a 10% dissipation rate per day, once daily shower, and 2 hours exposure per day.

6.3.2 Results

Table 3 below presents the results of the PBPK model runs for each of the six exposure scenarios.

Table 3. Summary of PBPK Model Runs for Analysis of Validation of Columbia Study Blood Levels

Model Run	Daily Exposure Duration (hours)	Highest Peak Blood Concentration (pg/g)	24-Hour Blood Concentration (pg/g)	Lowest Peak Blood Concentration (pg/g)	10 Hours After the Last Peak on Day-30 Blood Concentration (pg/g)	24 Hours After the Last Peak on Day-30 Blood Concentration (pg/g)
Broadcast (Hard Floor)	2	7,179	41	396	42.6	31.7
Perimeter (Carpeted Floor)	8	1,053	13	63	12.9	10.7
60 pg/g Peak 30th Day	2	~ 1,030	4.3	60	6.44	4.79
	8		12.8		12.7	10.6
60 pg/g Peak on Day of Application	2	60	0.25	~ 3.5	0.37	0.28
	8		0.74		0.73	0.61

Broadcast (hard floor) and perimeter (carpeted floor)

- The broadcast and perimeter exposure scenarios result in the highest exposure potential of all scenarios modeled.
- The highest peak internal dose predicted for post-application exposures to broadcast (hard floor, 2 hours) applications is 7,179 pg/g. For the perimeter (carpeted floors, 8 hours) application, peak internal dose is 1,053. Peak internal dose predictions occurred on the day of product application.
- Internal dose drops rapidly following the highest peak resulting in predicted 24-hour internal dose (blood concentrations) of 41 and 13 pg/g for broadcast and perimeter model runs, respectively.
- Peak internal dose drops with each subsequent day until the lowest peak is attained on the final day of modeled exposure resulting in predicted internal doses of 396 and 63 pg/g for the broadcast and perimeter runs, respectively.
- Following the final day of modeled exposure, internal dose drops initially. Ten hours after the final day peak exposure, internal doses predicted from broadcast and perimeter exposures are 43 and 12.9 pg/g, respectively.
- Twenty-four hours following the final day of exposure predicted internal doses are 31.7 and 10.7 pg/g for the broadcast and perimeter runs, respectively.
- All peak levels predicted for the broadcast model run are above the 60 pg/g level. The 60 pg/g level is predicted for the perimeter model run on the 30th (final) day.

60 pg/g peak on the 30th day of exposure

- The highest peak internal dose predicted for post-application exposures from hard surface and carpet applications is 1,030 pg/g. Twenty-four hours later the internal dose rapidly declines to 4.3 and 12.8 pg/g for hard floor (2 hours) and carpeted floors (8 hours) exposures, respectively.

- As designed for the simulation, the (lowest) peak internal dose of 60 pg/g occurs on the final day of product application.
- Following the final day of modeled exposure, internal dose drops initially. Ten hours after the final day peak exposure, internal doses were of 6.44 and 12.7 pg/g hard floor (2 hours) and carpeted floors (8 hours) exposures, respectively.
- Twenty-four hours following the final day of modeled exposure the predicted internal doses are 4.79 and 10.6 pg/g for the hard floor (2 hours) and carpeted floors (8 hours) exposures, respectively.

60 pg/g peak on the day of application

- As designed for the simulation, the peak internal dose of 60 pg/g occurs on the day of product application. Twenty-four hours later the internal dose rapidly declines to 0.25 and 0.74 pg/g for hard floor (2 hours) and carpeted floors (8 hours) exposures, respectively.
- The lowest peak internal dose, ~3.5 pg/g, occurs for both the broadcast and perimeter runs on the final day of modeled exposure.
- Following the final day of modeled exposure, internal dose drops initially. Ten hours after the final day peak exposure, the internal doses of 0.37 and 0.73 pg/g are predicted for hard floor (2 hours) and carpeted floors (8 hours) exposures, respectively.
- Twenty-four hours following the final day of modeled exposure the predicted internal doses are 0.28 and 0.61 pg/g for the broadcast and perimeter runs, respectively.

Figures 6 through 8 consistently show a saw-tooth pattern of daily, rapid increases in internal dose during the exposure period followed by a rapid decline after the exposure period. The peak internal dose for all modeled runs occurred during the final hour of daily exposure, just prior to the showering event. A rapid drop in internal dose is seen following the bathing/showering event and continues to decline until 24 hours following the prior day's exposure, just before initiation of the exposure event for the subsequent day. Internal dose increases once again immediately following initiation of the exposure event.

Further, internal dose peaks on the initial day of exposure and a step wise decline can be observed with each subsequent day modeled. This effect is due to the assumption of 10% daily dissipation of chlorpyrifos residue. Thus, the potential for exposures to chlorpyrifos are reduced with each subsequent day modeled.

Figures 6 through 8 consistently show that the width of within day internal dose varies based on the duration of daily exposure — 2-hour exposure to hard surfaces or 8-hour exposure to a carpeted surface. The simulations of the 2-hour daily exposure duration results in a narrower width than when compared to the 8-hour exposure duration.

A rapid decline can be observed for all figures presented below (6–8) following the 30th (final) day of exposure. This decline continues to a horizontal asymptote.

Figure 6. Graphical representation of the PBPK model run conducted for estimated post-application exposures following perimeter application to carpeted floors, 8 hours exposure duration (left) and broadcast application to hard floors, 2 hours exposure duration (right) using Residential SOP algorithms and inputs.

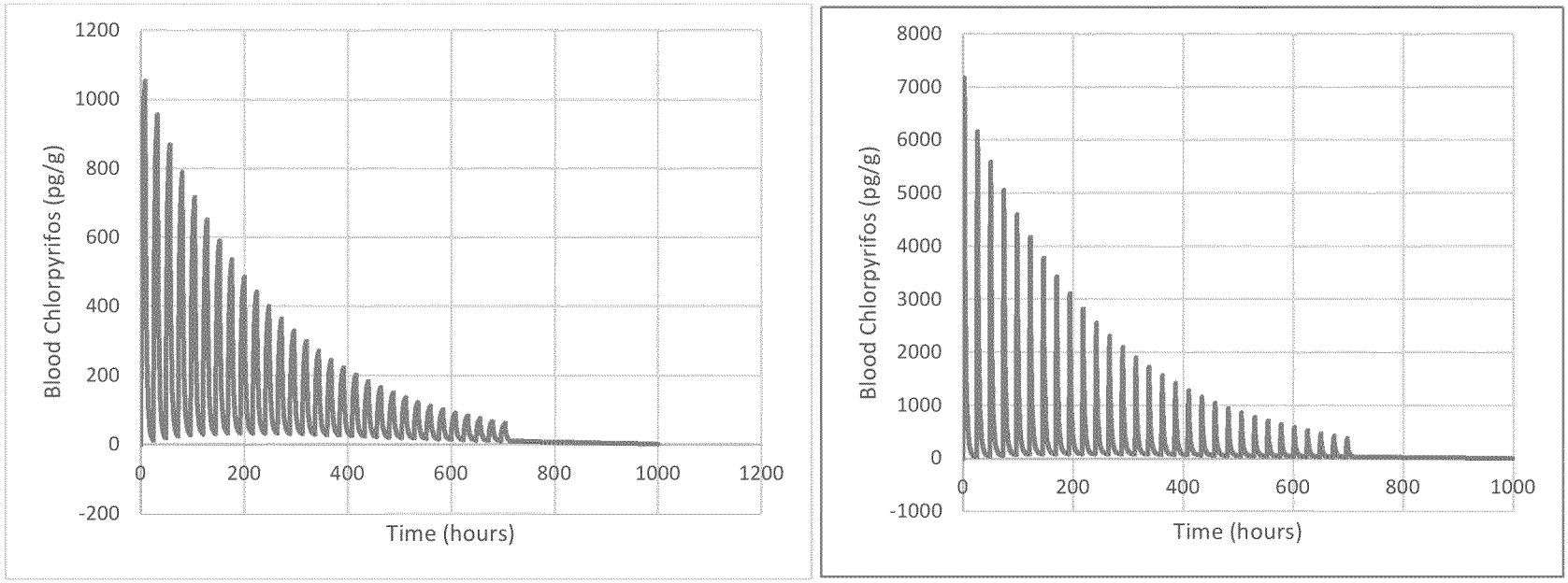


Figure 7. Graphical representation of the PBPK model run conducted for evaluation of the peak blood chlorpyrifos concentration on the initial day of exposure assuming a peak of 60 pg/g on the final day (30th) of indoor exposure; 8 hour exposure duration (left) and 2 hour exposure duration (right).

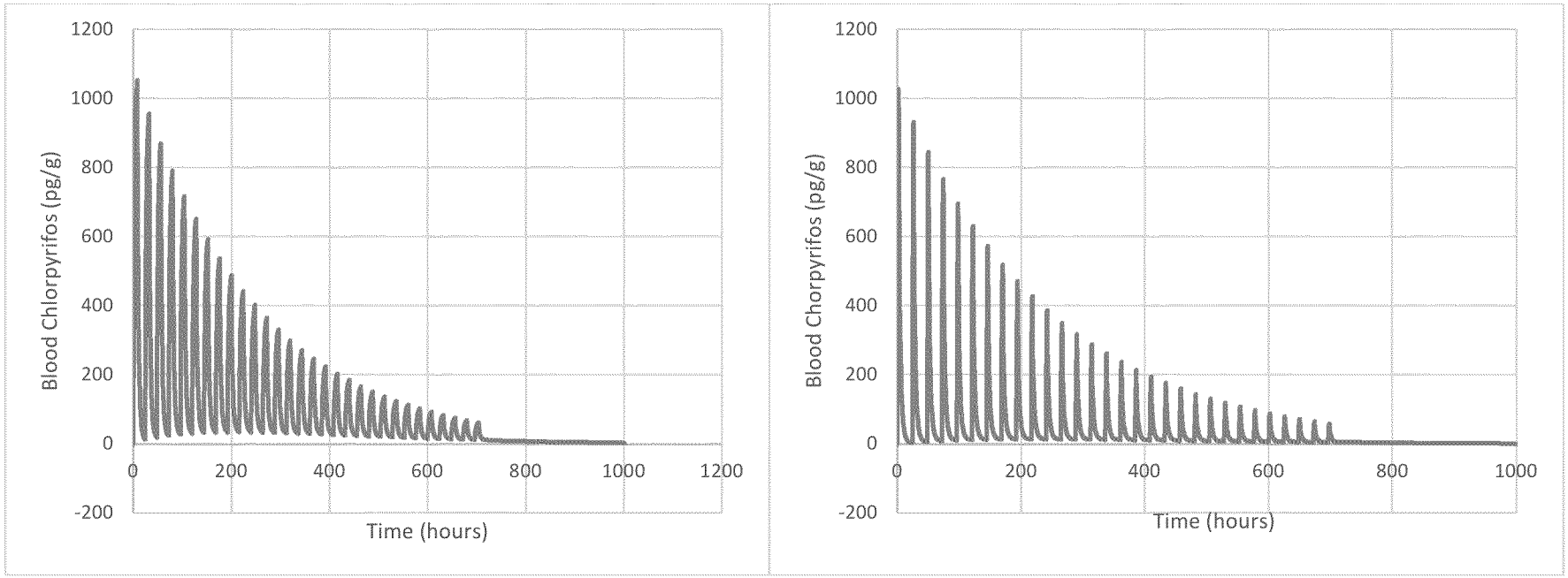
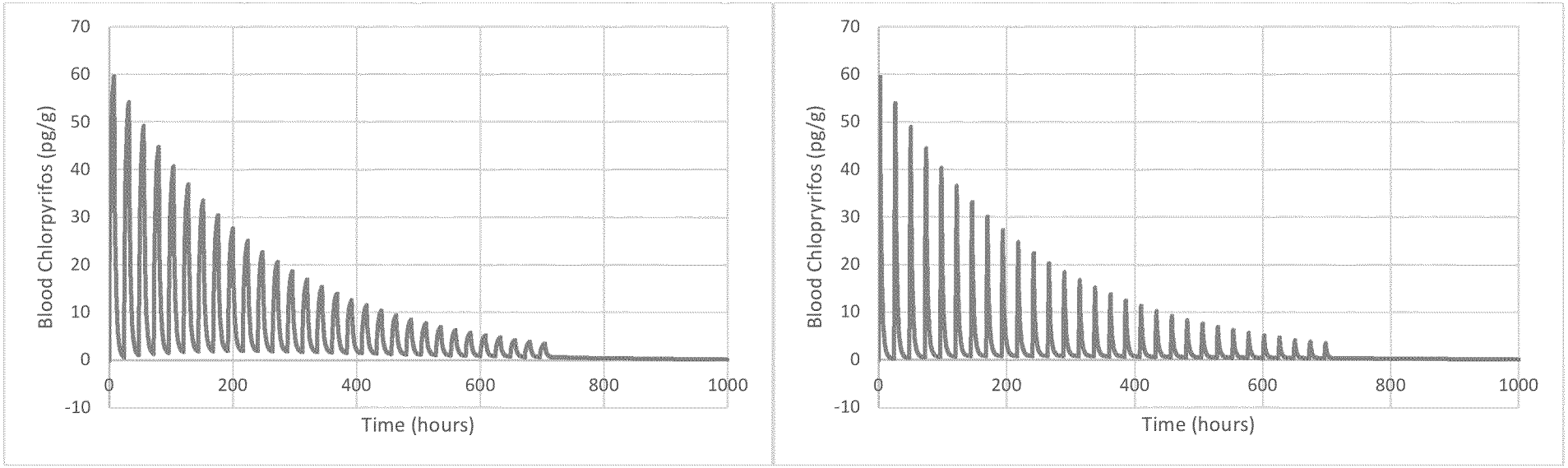


Figure 8. Graphical representation of the PBPK model run conducted for evaluation of peak blood chlorpyrifos concentrations on the final day of exposure (30th) assuming an initial blood concentration of 60 pg/g; 8 hour exposure duration (left) and 2 hour exposure duration (right).



6.3.3 Discussion of Residential Exposure Characterization

Table 3 and Figures 6 through 8 above provide the results of the six post-application exposure scenarios, which mimic indoor chlorpyrifos usage by the CCCEH cohort and were input into the PBPK model to predict the corresponding internal dosimetry.

The two residential exposure scenarios, broadcast (hard surface) and perimeter (carpet), were evaluated with use of the algorithms and inputs described in the 2012 Residential SOPs. The estimated exposures and corresponding internal doses predicted with use of the PBPK model are well within the range of the levels reported in the CCCEH cohort and, suggest that the measured blood levels were likely from chlorpyrifos use in the home environment. The internal doses estimated for broadcast and perimeter applications are below 60 pg/g in as little as 24 hours following the initial (peak) day of exposure, and drop rapidly within the first 10 and 24 hours after the final day peak exposure. This observation supports that the women in the CCCEH cohort could have had internal dose levels well within the high range of those reported on any given day following monthly treatment. Further, for the perimeter (carpet) exposure scenario, the estimated peak internal dose on the 30th (final) day of exposure is approximately 60 pg/g. That is, even in consideration of perimeter carpet peak internal dose, levels in the range of the 60 pg/g following exposures of this magnitude are attainable.

Figure 1 provides a summary of the reported blood levels in CCCEH across the cohort by year; this information was provided to the agency in 2015. The 1998–1999 CCCEH data represent the time period of highest use during the epidemiology study. For the 1998–1999 CCCEH data, the 75th and 95th percentile are reported as 9.55 pg/g and 19.35 pg/g, respectively (Figure 1). These values are similar to the agency's estimates at 24 hours following the final day of peak exposure for the perimeter carpet (10.7 pg/g) and broadcast hard surface (31.7 pg/g) scenarios (Table 3). At the 24-hour time point following the final day of peak exposure, the perimeter carpet (10.7 pg/g) simulation is approaching the lower end of the top tertile used in Rauh *et al.* (6.17 pg/g; 2006, 2015). Moreover, the pattern of internal doses modeled for the perimeter (carpet) exposure scenario and that predicted for simulation using 60 pg/g as the peak at day 30 are very similar including the maximum peak, lowest peak, the 24-hour blood concentration following peak exposure on day of an application, and the 10- and 24-hour blood concentrations after the final day peak exposure for 8 hours of exposure. The results of these simulations together support the conclusion that the reported values in the CCCEH are driven primarily by residential use of the broadcast and perimeter chlorpyrifos products. These results further support the reasonableness of the magnitude and distribution of data reported data by the CCCEH.

For the simulations where 60 pg/g is the peak at the beginning of the simulation (*i.e.*, day of application), the low values after the showering events are at or below the LOD (0.5 or 1.0 pg/g) at all time points. This pattern is more reflective of the CCCEH in years after the cancellation of the indoor uses and not reflective of the residential use. For example, in the 1998/1999 CCCEH

data, even at the 10th percentile, the maternal blood data are above the LOD. In contrast, beginning in 2001, there is a dramatic increase in the number of samples below the LOD (Figure 1) with only the 75th percentile and higher with reported values above the LOD. These results may suggest that this scenario is too low to represent actual residential exposure.

In sum, the purpose of these exposure scenarios was to characterize the blood levels of chlorpyrifos reported by CCCEH investigators. Even using EPA's Residential SOPs with high end assumptions for broadcast use on hard surface (the highest possible scenario), 24 hours after peak exposure on day 1 of an application, 10 and 24 hours after the final day peak exposure the predicted values are within the range of blood values reported by CCCEH. Lower internal doses are achieved with lower but still high end exposure scenarios (perimeter use on carpet) and lead to values in the terminal half-life phase near the low end of top tertile values in the CCCEH (6.17 pg/g) and near the 75th percentile of the maternal blood levels for the 1998/1999 time period when chlorpyrifos use was highest for the cohort. Taken together, this exposure characterization analysis suggests that the CCCEH values are plausible and reasonable based on the agency's analysis and likely driven by residential exposure. Food exposure is expected to have occurred but not likely lead to higher levels in blood whereas drinking water exposure was unlikely.

7.0 Deriving a Point of Departure (PoD) for Neurodevelopmental Outcomes

7.1 Uncertainties with Using Biomarker for the PoD

In CCCEH, chlorpyrifos in blood was used as the exposure metric to evaluate the association between chlorpyrifos exposures and adverse health outcomes. While biomarker data are arguably superior to conventional exposure data in that they reflect chemicals that were absorbed in the body from all routes and sources, they do not provide direct measure of environmental exposure levels important for regulatory purposes. In general, biomarkers are expected to reflect aggregate exposures over a period less than five half-lives prior to sampling. For chemicals with short biological half-lives such as chlorpyrifos, their biomarker levels are determined by recent exposures. Especially for chlorpyrifos in blood, it is a snapshot of the instantaneous, internal concentration at time of sampling. Depending on the exposure patterns, there can be large intra-individual temporal variability in spot measurements of blood concentration. When one attempts to use biomarker measurements as an exposure surrogate to evaluate the association between exposure and disease, the assumption is that higher biomarker concentrations came from higher exposure concentrations. But, it is not difficult to see that this assumption could be questioned in CCCEH by randomly selecting a time point on the PBPK model-simulated time course of chlorpyrifos concentrations in blood under various exposure scenarios to simulate a spot blood measurement in CCCEH. A specific blood level may be reached immediately after the termination of exposure at a lower dose, several days after the termination of exposure at a higher dose, or any combinations of dose and timing of exposures. Without knowing the timing of exposures relative to blood sample collection, a single measure of chlorpyrifos concentration

in blood was only evidence of exposure and absorption, and cannot be used to reconstruct sources, routes, frequency, duration, timing, and magnitude of exposures. For these reasons, the agency has not attempted to conduct reverse dosimetry to estimate doses that could have resulted in these observed blood levels, but has instead opted for conducting forward dosimetry through the development of a series of likely exposure scenarios that span a broad range of exposure potential to compare the predicted blood concentrations of chlorpyrifos with data observed in CCCEH (Section 6.3).

With the proposed approach, the assumption is that the CCCEH cord blood data were not collected during the period at which blood concentrations of chlorpyrifos were at their peak or rapidly changing, but instead at or near the low points on PK curves and likely during the asymptote period where blood concentrations were fairly stable across several days. Cord blood was collected at delivery which suggests that volunteers in CCCEH have spent hours in labor and delivery in the hospital, meaning removal from their apartments and the exposure to chlorpyrifos had ceased.

Besides the large uncertainty associated with linking chlorpyrifos concentrations in blood to chlorpyrifos doses being exposed by these volunteers, uncertainty also exists when establishing a quantitative relationship between chlorpyrifos concentrations in blood and adverse health outcomes. Ideally, such relationship can only be established when a biomarker is a reasonable surrogate for the toxic moiety at the target tissue. For health endpoints investigated in these epidemiologic studies, the adverse outcome pathways, toxic moieties, and biological targets were all unknown. Furthermore, it is always challenging in epidemiological studies to perform meaningful analysis to link biomarker measurements that reflect short-term exposures to long-term health outcomes. The key assumption is that measured biomarker levels reflect exposures during time windows that were critical for disease onset. It is also not clear whether cord blood concentrations measured at birth reflect exposure levels during the critical time window(s). However, there is a reasonable likelihood that chlorpyrifos was applied multiple times in the apartments of the women in the cohort over the course of the pregnancy (potentially once a month) increasing the potential for exposure during those unknown critical period(s).

In the context of the uncertainties associated with using the CCCEH blood data in quantitative risk assessment, there is concern that the PoDs based on AChE inhibition (Appendix 1) may not be adequately protective of human health. For example, given an external dose required to achieve 10% AChE inhibition for a female worker who was exposed dermally to chlorpyrifos 8 hours/day, 5 day/week for 3 weeks, the blood concentration of chlorpyrifos peaked at 120,000 pg/g, and was still above 100 pg/g at 32 days after the last exposure. Similarly, at a food exposure level leading to 10% AChE inhibition, chlorpyrifos concentration in blood never goes below 100 pg/g over the continuous 21-day exposure simulation and is around 7000 pg/g at the daily peaks. The agency could consider continuing to use the AChE PoDs, but additional safety factors beyond the FQPA Safety Factor 10X would be needed to have sufficient confidence to

conclude that there is a reasonable certainty of no harm under the FQPA. However, the agency would still need to quantify such additional factors—and the analysis to quantify them would again require the agency to make quantitative use of the CCCEH cord blood data with the same uncertainties described above. Although either approach would be possible, the agency has elected to propose to use the cord blood directly as the PoD as the simpler, more understandable approach.

7.2 Options for PoD Based on the CCCEH Biomonitoring Data

From the CCCEH publications, there are two general options for deriving a PoD for extrapolating neurodevelopmental risk from chlorpyrifos exposure.

1) ***Lower limit of the top tertile (>6.17 pg/g cord blood) derived from Rauh et al. (2006) and repeated in other CCCEH publication.*** Rauh *et al.* (2006) includes 254 children followed through age 3 years and used a dichotomized statistical approach of <6.17 pg/g cord blood for the 'low group' and >6.17 pg/g cord blood as the 'high' group. Using the same CCCEH study data, earlier studies (Whyatt *et al.*, 2004; Perera *et al.*, 2003) also documented statistically significant associations between birth outcomes and cord blood chlorpyrifos levels (>6.17 pg/g); however, these birth outcome associations are not the focus of this assessment. The Rauh *et al.* (2006) study reported statistically significant deficits of 6.5 points on the Bayley Psychomotor Development Index (PDI) at 3 years of age when comparing high to low exposure groups. Notably these decrements in PDI persist even after adjustment for group and individual level socioeconomic variables (Lovasi *et al.*, 2011), with this follow-up study using the same dichotomized statistical approach for grouping high and low exposure. These investigators also observed increased odds of mental delay (OR=2.4; 95% CI: 1.1–5.1) and psychomotor delay (OR=4.9; 95% CI: 1.8–13.7) at age 3 years when comparing high to low exposure groups (Rauh *et al.*, 2006). Rauh *et al.* (2006) also reported extremely large odds ratios for attention disorders (OR=11.26; 95% CI: 1.79–70.99), ADHD (OR=6.50; 95% CI: 1.09–38.69), and PDD (OR=5.39; 95% CI: 1.21–24.11) at age 3 years when comparing high to low chlorpyrifos exposure groups (Rauh *et al.*, 2006). Furthermore, these investigators observed increased odds of mild to moderate tremor at age 11 when comparing high to low exposure groups (Rauh *et al.*, 2015). As such, if the agency pursued these studies for deriving a PoD, the appropriate value would be 6.17 pg/g cord blood.

2) ***Benchmark Dose estimates derived from linear regression reported in Rauh et al. (2011) for deficits in Working Memory.*** Rauh *et al.* (2011) evaluated the relationship between prenatal chlorpyrifos exposure and neurodevelopment among 265 of the CCCEH cohort participants who had reached the age of 7 years and had a complete set of data including prenatal maternal interview data, prenatal chlorpyrifos marker levels from maternal and/or cord blood samples at delivery, postnatal covariates, and neurodevelopmental outcome data. Rauh *et al.* (2011) described the log of Working Memory Index (WMI) of children as linearly associated with concentration of chlorpyrifos (CPF) in cord blood with a slope = -0.006

(95% CI = -0.01, -0.002). Investigators reported that for each standard deviation increase in exposure (4.61 pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.

For deriving potential benchmark dose (BMD) estimates from Rauh *et al.* (2011), the agency is interested in estimated concentration levels of chlorpyrifos in cord blood associated with specific percent(s) reduction in WMI of children. The concentration levels of chlorpyrifos in cord blood (and its 95% lower confidence limit, one-sided) can be estimated using the estimated slope and its 95% lower confidence limit (one-sided). However, neither the 95% lower confidence limit nor the standard error of the estimated slope (which can be used to calculate the 95% lower confidence limit) were available in Rauh *et al.* (2011). Therefore, the standard error of estimated slope was back-calculated as: (estimated slope – lower bound of 95% CI)/1.96 which was then used to calculate the 95% lower limit (one-sided) of estimated chlorpyrifos levels. The justification of using the 95% lower confidence limit (one-sided) of an estimated slope to estimate the 95% lower confidence limit of a dose level is described in Appendix 5 and confirmed by a simulation. The SAS code of the simulation is included in Appendix 5.

From the estimated slope = -0.006 and its 95% (two sided) CI = (-0.010, -0.002) from Rauh *et al.* (2011), the 95% lower confidence limit (one-sided) of the estimated slope was calculated as described above to be -0.009. Using the values of estimated slope and its 95% one-sided lower confidence limit, the chlorpyrifos concentrations and their 95% lower limit associated with 1%, 2%, 3%, 4% and 5% reduction in WMI were calculated. The results of calculations are presented in Table 4 below.

In order to make a PoD proposal based on the results presented in Table 4, the agency took several factors into consideration using a bounding approach. First, at the lower end, a 1% change in working memory is predicted which corresponds to the central and lower limit estimates of the internal dose (as indicated by cord blood levels) of 1.68 and 1.07 pg/g, respectively. These values are close to the LODs of 0.5/1.0 pg/g for measuring chlorpyrifos residues in cord blood (Rauh *et al.*, 2006; 2011; 2015). Because of the proximity to the LOD, the lower cord blood levels have more uncertainty associated with them than higher cord blood levels (e.g., associated with a ≥2% loss in working memory) because they likely are based on partially imputed data and are thus not completely quantitatively supported. Second, at the higher end, there is a basis for the upper limit bound levels at which chlorpyrifos exposure has been associated with neurodevelopmental effects, corresponding to chlorpyrifos levels in cord blood that were evaluated in Rauh *et al.* (2006, 2015). Specifically, the estimated blood concentrations to achieve 3–5% reductions in working memory are near or above the 6.17 pg/g which represents the lower limit of the top tertile where neurodevelopmental outcomes have been associated with chlorpyrifos exposure (Rauh *et al.*, 2006, 2015). As such, selecting a PoD based on a WMI ≥ 3% which occurs at a cord blood BMD of 5.08 pg/g (BMDL 3.26 pg/g) may not be protective of such outcomes. Therefore a 2% working memory reduction which

corresponds to an internal dose measure of 2.16 pg/g chlorpyrifos in cord blood is quantitatively near the value reported by Rauh (2.8% reduction in working memory) and within the range of levels measured in the blood samples collected by investigators. Thus, a value of 2% is proposed as the PoD which is supported by the existing data and it is health protective. Finally, it should be noted as a matter of science policy when using BMD estimates for deriving PoDs, the agency uses the lower limit (BMDL), not the central estimate (BMD) (USEPA, 2012c). As such, selection of the 2.16 pg/g value is consistent with how BMDLs are generally considered in agency risk assessments (*i.e.*, since it is the BMDL and not the BMD). ***In summary, the agency is proposing to use the 2% reduction as the PoD in working memory or an internal dose of 2.16 pg/g.***

Table 4. Estimated concentration of CPF in cord blood at specific percent reduction of WMI

Estimated slope[a] (95% CI, two-sided)	Calculated by EPA\HED			
	95% lower confidence limit (one-sided) of estimated slope[b]	Percent Reduction of WMI (%)	Estimated concentration of CPF in cord blood[c] (pg/g): BMD	95% lower limit (one-sided) of estimated concentration of CPF in cord blood[d] (pg/g): BMDL
-0.006 (-0.010, -0.002)	-0.009	1	1.68	1.07
		2	3.37	2.16
		3	5.08	3.26
		4	6.80	4.36
		5	8.55	5.48

[a] estimated slope (95% CI) was as stated in Rauh *et al.*, 2011 study
[b] 95% lower confidence limit (one sided) of the estimated slope calculated by HED, using the Rauh's estimated slope and its 95% CI to estimate SE
[c] estimated CPF concentration associated with the given percent of reduction in WMI, calculated using the estimated slope = -0.006
[d] 95% lower confidence limits of CPF concentrations associated with the given percent of reduction in WMI, calculated using the 95% lower confidence limit = -0.009 of the estimated slope

8.0 Assessing Extrapolation/Uncertainty

No risk assessment can reflect risk with absolute certainty, so it is important that uncertainties be accounted for in a predictable, scientifically defensible manner that is both consistent with EPA's policies and responsive to the needs of decision makers (U.S. EPA, 2004). In deriving reference concentrations (RfCs) and reference doses (RfDs), the agency has historically used default uncertainty factors (UFs) to compensate for a lack of information (U.S. EPA, 2002).

In typical risk assessments, PoDs are derived directly from laboratory animal studies and inter- and intra-species extrapolation is accomplished by the use of 10X factors. In the case of chlorpyrifos, the proposed PoDs are derived from human information obviating the need for the inter-species extrapolation. The intra-species uncertainty factor (UF_H) is applied to account for variations in susceptibility within the human population (human variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure response- relationship in the groups of the human population that are most sensitive to the health hazards of the chemical being assessed (U.S. EPA, 2002). As such, the agency still needs to consider intra-species extrapolation of the PoD from the CCCEH epidemiology data across the diverse human population. Moreover, the agency must consider the statutory requirement of the FQPA 10X Safety Factor for "**potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children**." An evaluation of strengths and uncertainties of the existing chlorpyrifos database of epidemiology and laboratory studies associated with the intra-species extrapolation factor and the FQPA 10X Safety Factor are provided in Section 8.2.

For the evaluation of strengths and uncertainties associated with the existing epidemiology database, OPP first considered existing agency experience in using epidemiology studies to evaluate neurodevelopment in children. Specifically, OPP has used the 2000 NRC report on methyl mercury and the methyl mercury IRIS entry[16] as a starting point for considering the key factors in evaluating uncertainty in the PoD and extrapolation. Table 5 was extracted from the 2000 NRC report on methyl mercury and provides the key characteristics of importance considered for the intra-species factor. Appendix 7 provides a comparison of the major considerations from NRC for methyl mercury with the strengths and uncertainties associated with the CCCEH epidemiology studies.

[16] http://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=73

Table 5. Table 8-2 extracted from the 2000 NRC report on methyl mercury.

TABLE 8-2 Sources of Uncertainty in Key Epidemiological Studies

Susceptible subpopulations

- Interindividual toxicokinetic variability in dose reconstruction
- Toxicodynamic variability
- Nutritional deficits

Measures of exposure

- Lack of dietary-intake data
- Extrapolation from biomarker Hg content to MeHg intake
- Nutritional and dietary confounders and effect modifiers
- Co-exposure to other neurotoxicants (e.g., PCBs)
- Co-exposure to other forms of Hg
- Inability to measure peak exposures
- Temporal matching of exposure to critical periods of susceptibility for the developing fetal brain

Lack of consideration of other key or most-sensitive health end points

- Potential cardiovascular or immune-system effects
- Neurological sequelae (i.e., late emerging effects)

8.1 Intra-species Extrapolation

The default value for UF_H is 10-fold and can be apportioned into pharmacodynamic (PD) and PK components values, often at one half order of magnitude each.

Pharmacodynamics (PD): The AOP(s) for neurodevelopmental outcomes related to chlorpyrifos exposure is/are not delineated such that the molecular initiating event(s) and related key events are not known nor is the quantitative dose response relationships of such events. By extension, the window(s) of susceptibility and associated duration(s) of exposure are not known. The SAP also noted similar uncertainty commenting on the "Lack of specificity of a critical window of effect and the potential for misclassification of individual exposure measures." Furthermore, given that biomarker data were taken at a single point in time (cord blood at delivery) and that chlorpyrifos exposure in the home was dynamic, there is uncertainty as to the representativeness of cord blood data for the entire pregnancy or for critical windows of susceptibility. On this issue, the SAP noted that with respect to "Use of a single or average sample for exposure… Although Whyatt *et al.* (2009) noted moderate but significant correlations between meconium and cord and maternal blood and average urine TCPy, the representativeness of a single point exposure is still unclear. Time-varying exposures or the ability to define cumulative exposures would be preferable." Similar uncertainties were noted in the methyl mercury studies with respect to unknown windows of susceptibility and the degree to which existing biomonitoring

data do or do not represent those critical periods. Moreover, given that the CCCEH investigators did not collect information on timing or frequency of chlorpyrifos applications, it is not known whether the existing cord blood encompasses the levels of chlorpyrifos near the day(s) of application(s).

The SAP noted an uncertainty associated with "External generalizability of the cohorts given their unique racial/ethnic and socioeconomic characteristics." The CCCEH study focused exclusively on women who self-identified as African-American or Dominican, were 18–35 years of age, and who lived in specific urban areas (northern Manhattan (Central Harlem or Washington Heights/Inwood) or the South Bronx) for a year or more prior to pregnancy. This study population was of generally low socioeconomic status, with approximately 85% of the population having annual incomes of $30,000 or less (Whyatt *et al.*, 2003). However, the agency does not have adequate data to assess the relative sensitivity of these groups compared with others. Given the current state of knowledge, the PD variability cannot be quantified and is therefore unknown. For PD variation, a data-derived factor cannot be derived and the default 3X is retained.

Pharmacokinetics (PK): With respect to PK variation, although there is a robust PBPK model which accounts for population variability for infants, a fully vetted, validated PBPK model is not available for pregnant women (See Section 4.0). As such, for PK variation, a data-derived factor cannot be derived and the default 3X is retained.

Intra-species Factor: As such for chlorpyrifos, the agency proposed to use a 10X intra-species extrapolation factor. This 10X, apportioned equally between 3X for PK variability and 3X for PD variability is consistent with that used for methyl mercury (Table 6).

Table 6. UFs recommended by NRC and used by EPA IRIS for methyl mercury

Consideration	Consideration from NRC Report	Used by EPA in IRIS Assessment
Pharmacokinetics	Interindividual toxicokinetic variability in dose reconstruction	Pharmacokinetic variability and uncertainty in estimating an ingested mercury dose from cord-blood mercury concentration: a factor of 3 was applied: NRC analyses of variability in the pharmacokinetic factors underlying the conversion of a biomarker level of methylmercury to an ingested daily dose of methylmercury that corresponds to that level
Pharmacodynamics	N/A	Pharmacodynamic variability and uncertainty: a factor of 3 was applied: the population of the Faroe Islands is descended from Scandinavian stock that settled many generations ago, and is extremely homogeneous. The average toxicodynamic response of this population compared with that of the United States, which is genetically much more diverse, is unknown. Similarly, the relative variability of different populations also is unknown. A threefold UF for toxicodynamic variability and uncertainty was applied.
Database Uncertainty	Data-base insufficiency (i.e., because of consideration of possible low-dose sequelae and latent effects, and immunotoxicity and cardiovascular effects)	N/A
Total UFs	"the committee believes that an overall uncertainty-factor adjustment of no less than 10 is necessary and appropriate to provide an adequate margin of protection."	overall UF of 10

8.2 FQPA 10X Safety Factor for Infants & Children

The FQPA (1996) instructs EPA, in making its "reasonable certainty of no harm" finding, that in "the case of threshold effects, **an additional tenfold margin of safety** for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account **potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children**." Section 408 (b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children."

8.2.1 Pre- and Post-Natal Toxicity Database

Experimental Laboratory Animals: There are numerous animal studies in the literature (see Appendix 3) which vary in their study design but all involve gestational and/or early postnatal dosing with behavioral evaluation from adolescence to adulthood. The data provide support for the susceptibility of the developing mammalian brain to chlorpyrifos exposure through gestation and early in life, with adverse outcomes in several neurological domains including cognitive, anxiety and emotion, social interactions, and neuromotor function. The studies have not shown that any specific developmental period is critical overall to the long-term outcomes, since similar effects are observed with different exposure periods. For example, cognitive changes in one laboratory using a radial arm maze were observed following gestational and early, but not late, postnatal exposure (Aldridge *et al.*, 2005; Icenogle *et al.*, 2004; Levin *et al.*, 2002; Levin *et al.*, 2001), whereas other laboratories cognitive deficits in a Morris water maze were reported following both gestational and late-postnatal exposure (Billauer-Haimovitch *et al.*, 2009; Turgeman *et al.*, 2011). Likewise, some changes in anxiety and social behaviors were reported at both gestational and postnatal exposure periods. Overall, these data do not clearly show specific critical periods of exposure but support a conclusion that early life (pre- and post-natal) represent susceptible lifestages.

Epidemiology Studies: Numerous epidemiological investigations have observed a link between prenatal exposure to chlorpyrifos or OPs (measured as chlorpyrifos, TCPy, or DAPs) and adverse effects on neurodevelopment through age seven years, with additional more limited evidence up through approximately age eleven. As noted previously, for these epidemiology studies chlorpyrifos was only assessed directly in the CCCEH study (Rauh *et al.*, 2006, 2012, 2015), with the Mexican cohort study (Fortenberry *et al.*, 2014) assessing the chlorpyrifos metabolite TCPy, and the CHAMACOS cohort study (Eskenazi *et al.*, 2007) measuring both TCPy and DAPs. In contrast, all of the other epidemiology studies assessed only DAP exposure, and as noted previously these DAPs are metabolites of multiple OPs including chlorpyrifos.

The majority of epidemiological studies investigated only prenatal exposures. Specifically, with respect to biomarkers representing prenatal exposures, the three US cohorts (CCCEH, Mt. Sinai, CHAMACOS) each reported evidence of impaired mental and psychomotor development, albeit not consistent by age at time of testing (ranging from 6 month to 36 months across the three cohorts). Statistically significant or suggestive associations between chlorpyrifos or DAPs exposure and attentional problems/ADHD were reported by multiple prospective cohorts (Rauh *et al.*, 2006; Eskenazi *et al.*, 2007; Marks *et al.*, 2010; and Fortenberry *et al.*, 2014) with additional support from a case control study, Bouchard *et al.* (2010). In addition, the three US cohorts and the CHARGE study have reported suggestive or positive associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Shelton *et al.*, 2014; Eskenazi *et al.*, 2007; Furlong *et al.*, 2014). While these studies vary in the magnitude of the overall strength of association, they have consistently observed a positive association between OP exposure and ASD. Several studies have also documented statistically significant association between prenatal

DAP exposure and abnormal motor development in neonates (reflexes, Brazelton score or similar measure; Young *et al.*, 2005; Engel *et al.*, 2007; Zhang *et al.*, 2014). Finally, CCCEH, Mt. Sinai and CHAMACOS have reported an inverse relation between the respective prenatal measures of chlorpyrifos or DAPs and intelligence measures at age 7 years (Rauh *et al.*, 2011; Engel *et al.*, 2011; Bouchard *et al.*, 2011).

A smaller number of studies have assessed postnatal exposure to DAPs. Postnatal exposures have not been assessed in the CCCEH and Mt. Sinai studies (Rauh *et al.*, 2011; Engel *et al.*, 2011); as such, there are no studies included in this analysis which directly assessed the potential for postnatal chlorpyrifos exposure and associations with neurodevelopmental effects. However, given that the major source of exposure (residential use) was cancelled partway through the CCCEH study which substantially reduced and largely removed chlorpyrifos from the home environment, this limits the ability of this study to inform the impacts of long-term postnatal exposure to chlorpyrifos on neurodevelopment from the current uses of chlorpyrifos.

Postnatal exposure to DAPs has been assessed in the CHAMACOS cohort (Eskenazi *et al.*, 2007; Young *et al.*, 2005; Bouchard *et al.*, 2011) and three cross-sectional studies (Guodong *et al.*, 2012; Bouchard *et al.*, 2010; Oulhote and Bouchard, 2013). With the exception of Bouchard *et al.* (2010), no adverse neurodevelopmental associations were found between postnatal urinary metabolite levels and any of the developmental outcomes. Bouchard *et al.* (2010) looked at U.S. children age 8–15 years in the 2000–2004 National Health and Nutrition Examination Survey (NHANES), and observed a positive association between attention and behavior problems and total DAPs and DMAPs, but not DEAPs.

The agency is aware that postnatal exposure to DAPs has been assessed but not found to be associated with neurodevelopmental outcomes in the CHAMACOS cohort. However, in sum, *given that the extensive experimental laboratory animal database suggests that the post-natal period is a potential susceptible time, the lack of postnatal exposure assessment in the CCCEH and Mt. Sinai studies is a source of uncertainty in the epidemiology database*.

8.2.2 Impact of Sample Size on CCCEH Findings

In the 2012 SAP report, the Panel noted the CCCEH studies had:

- "Relatively modest sample sizes which limited the statistical power to classify some meaningful differences as statistically significant and to examine the effect of modification by race/ethnicity and other characteristics."
- "Relatively moderate to large exposure differences needed to see significant effects, likely due to the modest sample sizes used."

Generally, lower sample sizes limit the statistical power of a study. This in turn may lead to larger and less stable confidence intervals and or error bars, including not achieving statistical significance. In EPA's analysis of the strengths and weaknesses of the epidemiology studies, it is apparent that while the chance that positive associations observed are false positives due to

systematic errors in the studies cannot be excluded, it is more likely that error present in these studies would lead to the under-estimation of the true association. Therefore, while alternative explanations for positive association can be hypothesized (*e.g.*, additional unmeasured or poorly measured positive confounding variables), these explanations are less plausible than the alternative that associations have been missed or under-estimated due to non-differential measurement error and low sample size across exposure strata. Exposure measurement error has been shown to more greatly influence epidemiology study results than unknown or unmeasured confounding variables. Additionally, the elimination of temporal bias due to the prospective study design employed within each of the three children's health cohorts assures that prenatal exposures preceded neurodevelopmental outcomes measured at birth, and in early and later childhood through age 7 years. There is additional, more limited, evidence of neurodevelopmental outcomes in later childhood through approximately 11 years from the Mt. Sinai and CCCEH studies (Furlong *et al.*, 2014; Rauh *et al.*, 2015). The strength of the associations measured was in some instances quite strong. For example, in relation to pre-natal chlorpyrifos exposure, measures of mental and psychomotor delays were 2–4 fold greater among the most highly exposed as compared to those in the lower exposure category, and the relation between attention-deficit symptoms (age three years) and *in utero* exposure was significantly elevated, although imprecise (Rauh *et al.*, 2006). However, associations in many instances were weak to moderate, possibly due to exposure measurement error such as timing of exposure in relation to cord blood collection.

Associations with neurodevelopmental outcomes were consistently identified with respect to the number of abnormal reflexes in the neonatal period, the presence of mental and behavioral issues as well as gross motor delays were pronounced especially in later toddler years of 24–36 months, and the observation of intelligence decrements were seen across the three cohorts using different measures of prenatal chlorpyrifos exposure. However, with regards to dose-response, the modest sample size in the CCCEH study make it difficult to say that the dose-response relationship between chlorpyrifos and neurodevelopmental outcomes in the overall U.S. population has been fully characterized. The magnitude of the PoD in the general U.S. population of infants and children may be higher or lower than that estimated using the CCCEH study results, and the shape of the dose-response curve may also be different.

8.2.3 Conclusion on the FQPA Safety Factor

The FQPA (1996) requires that the agency include "an additional tenfold margin of safety" unless "on the basis of reliable data, such margin will be safe for infants and children." In other words, the FQPA 10X Safety Factor is present unless there are reliable data to remove it. In the case of chlorpyrifos, there are remaining uncertainties surrounding 1) the lack of information on post-natal exposure and evaluation of such exposure to neurodevelopmental outcomes in the CCCEH and Mt. Sinai cohorts and 2) the modest sample sizes which limited the statistical power and exposure differences required to see statistically significant results. As such, there is sufficient uncertainty which prevents the agency from reducing or removing the statutory 10X

FQPA Safety Factor. EPA therefore intends to continue to retain **the FQPA 10X Safety Factor for assessing risk from chlorpyrifos for infants, children, youths, and women of childbearing age for all exposure scenarios.**

9.0 Proposed Approach to Deriving Internal Dose Estimates: Integration of Exposure Assessment & PBPK Modeling

9.1 Overview

Typical risk assessments use administered dose as the PoD from an animal study, or less often, human data and then compare PoDs to external exposure levels to estimate health risks result from exposures. As such, risk metrics most often used by OPP to assess human health are the reference dose (RfD) and margins of exposure (MOE)[17]. For the chlorpyrifos risk assessment, the agency is proposing to use internal blood concentrations of chlorpyrifos derived from the CCCEH cord blood data as the PoD (Section 7.0). Because of the uncertainties associated with reverse dosimetry due to unknown exposure timing, frequency, and magnitude, the agency cannot confidently derive an administered/external dose from the CCCEH data. As such, the agency must use forward dosimetry to derive internal concentrations of chlorpyrifos from the most likely exposure scenarios to compare with the internal PoDs. Given the same exposure scenario (*e.g.*, single daily oral dose, 2 hours/day dermal dose for 30 days), the internal concentrations are linearly related to external doses.

Specifically, the agency has proposed an internal concentration PoD of 2.16 pg/g adjusted by 100X based on the intra-species factor and 10X FQPA Safety Factor. **As such the internal concentration RfD for assessing dietary (food and water) exposure is 0.022 pg/g. For exposure assessment which uses MOE as the risk metric (residential, occupational assessment), the internal PoD of 2.16 pg/g will be used with a target MOE of 100.**

As described earlier, the PK profile for chlorpyrifos in blood is characterized by rapid increases and decreases during exposure periods and immediately after exposure, followed by slower decrease during the terminal clearance phase. Because the CCCEH cord blood data are likely to represent data from the low points on the PK profile and likely in terminal clearance phase, the agency will, to the extent possible, compare internal concentrations of chlorpyrifos at 10 and 24 hours after the last peak in each simulation, specifically for food exposure and occupational/residential exposure scenarios.

Drinking water exposure provides a different challenge as exposure is more frequent. In particular, infants feed multiple times per day and night for several months. For these pathways,

[17] RfD = Point of Departure/ Uncertainty Factors and then is compared to exposure ; MOE = Point of Departure /Exposure and then is compared to total uncertainty factors

the agency is comparing the internal PoD and internal RfD to the full range of potential internal exposures.

The following sections provide overview information on OPP's exposure assessment approaches for food, water, and occupational/residential exposure assessment followed by case study examples of inputing OPP exposure estimates into the PBPK model for women of childbearing age and infants.

9.2 Food Exposure

9.2.1 Methods

EPA's OPP has performed acute and 21-days and longer [*i.e.*, steady state dietary (food)] exposure assessments for chlorpyrifos. This case study was performed for the purpose of obtaining estimated food exposure values for critical lifestages of concern (infants and females of childbearing age) for use with the chlorpyrifos PBPK model and were originally performed as part of the 2014 HHRA (USEPA, 2014a). The analyses considered combined exposure from all food commodities from crops or livestock for which there is a current U.S. tolerance for chlorpyrifos. The methods used are summarized below as are the resulting dietary exposure estimates for infants (<1 year old) and females (13–49 years old). The dietary assessment methods described below have undergone previous FIFRA SAP review (FIFRA SAP 2000a; FIFRA SAP 2000b) and are not subject to the current SAP review but are presented for purposes of assessing the new approach to integrate the exposure assessment with the PBPK model.

Based on evidence [food monitoring data, crop field trials and metabolism studies (Trichilo, 1988)] indicating that the metabolite chlorpyrifos oxon would be not be present in edible portions of the plant, it is not a residue of concern in food or feedstuff. The chlorpyrifos oxon, along with parent compound chlorpyrifos, is extensively tested for in a wide variety of commodities by the U.S. Department of Agriculture's (USDA's) Pesticide Data Program (PDP) food monitoring program. [18] While chlorpyrifos is frequently detected, the oxon is not; in fact, from 2007 to 2012, out of several thousand samples of various commodities, only one sample of potato showed presence of the oxon at trace levels, 0.003 ppm where the LOD was 0.002 ppm (even though there are no registered uses of chlorpyrifos on potato in the U.S.). The oxon metabolite was not found in milk or livestock tissues in cattle and dairy cow feeding studies, even at exaggerated feeding levels, and is not a residue of concern in livestock-based food commodities (Trichilo, 1988). The residue of concern included in the dietary exposure assessments is the parent compound chlorpyrifos.

Chlorpyrifos acute dietary exposure analyses were conducted for the 2014 HHRA using the Dietary Exposure Evaluation Model™ (DEEM) with the Food Commodity Intake Database

[18] https://www.ams.usda.gov/datasets/pdp

(FCID), Version 3.16, which incorporates 2003–2008 consumption data from USDA's National Health and Nutrition Examination Survey, What We Eat in America, (NHANES/WWEIA). [19] The data are based on the reported consumption of more than 20,000 individuals over two non-consecutive survey days. Foods "as consumed" (e.g., apple pie) are linked to EPA-defined food commodities (e.g., apples, peeled fruit — cooked; fresh or N/S; baked; or wheat flour — cooked; fresh or N/S, baked) using recipe translation files developed jointly by USDA/ARS and EPA. The diary is the entire list of foods consumed by one person on a single day. The weights of the food consumed in that day are multiplied by their respective pesticide residue values and added together to determine the quantity of pesticide consumed. That value is divided by body weight to arrive at the exposure estimate in terms of milligrams per kilogram body weight per day (mg/kg bw/day). Infants and children consume a greater quantity of food per unit body weight than adults. As a result, exposure estimates are generally higher for population subgroups comprised of infants and children.

Individual one-day food consumption data are used on an individual-by-individual basis. The reported consumption amounts of each food item can be multiplied by a residue point estimate and summed to obtain a total daily pesticide exposure for a deterministic exposure assessment, or "matched" in multiple random pairings with residue values and then summed in a probabilistic assessment. Residue distributions are made up of individual residue values that have been entered into files. These files are referred to as residue distribution files, or RDFs. When a distribution of consumption values is used, a distribution of exposure estimates is also obtained.

Chlorpyrifos steady state dietary exposure analyses were conducted for the 2014 HHRA using the Calendex-FCID™ program. Calendex provides a focus detailed profile of potential exposures to individuals across a calendar year. HED's steady state assessment considers the potential risk from a 21-day exposure duration using a 3-week forward rolling average (sliding by day) across the year. Like DEEM, the Calendex software uses the NHANES/WWEIA consumption data and pairs one diary for each individual in the WWEIA with a randomly selected set of residue values for each food consumed. The same food residue values used in the acute assessment using DEEM were used for the 21-day (steady state) duration using Calendex. Food residues are also input as either a single point estimate (deterministic) or as an RDF (probabilistic).

9.2.2 Food Residue Assumptions

Food tolerances are based on crop field trial data. Crop field trials are conducted at the maximum label rate and the minimum pre-harvest interval stipulated on the label. The use of field trial residues and tolerance level residues for foods in a dietary assessment represents conservative assumptions.

[19] http://www.ars.usda.gov/services/docs.htm?docid=13793

Food monitoring data are taken closer to the point of consumption than data from field trials. In addition, before analysis, food samples are prepared as if for consumption. As a result, they more adequately reflect residues present at the time foods are consumed. Residue monitoring data used in the chlorpyrifos dietary assessment were obtained from the USDA's Pesticide Data Program (PDP), which routinely tests for chlorpyrifos. For crops/foods not tested by PDP, translations have been made from tested crops (USEPA, 1999). Occasionally, older PDP data has been used where it represented the best estimate of real residues; the overall years of PDP data used in the chlorpyrifos assessment are from 1998 to 2012. Field trial data or tolerances have been used for a few crops where translations from PDP data were not appropriate.

Percent crop treated estimates for chlorpyrifos are provided by OPP's Biological and Economic Analysis Division (BEAD) in a screening level usage analysis (SLUA) report (USEPA, 2014b). Percent crop treated is the ratio of base acres of a crop treated with a pesticide to the total number of acres planted or grown, expressed as a percentage. The SLUA gives the average pounds of active ingredient used per year, the average %CT, the maximum %CT, and the sources from which the data were obtained. These sources include the U.S. Department of Agriculture's National Agricultural Statistics Service (USDA NASS), private pesticide market research data, and the California Department of Pesticide Regulation (DPR).

Percent CT information, where available for a given crop, is incorporated into the chlorpyrifos dietary exposure assessments. Percent CT estimates are used for the mathematical purpose of determining the numbers of zeros and ½LODs to be entered into the RDFs, or to be averaged into a point estimate. When a %CT estimate is used for a commodity, the blending classification of the commodity determines whether a point estimate or RDF is used for the commodity.

The maximum %CT estimates reported in the SLUA are used in the chlorpyrifos dietary assessments. Where no %CT data were reported for a commodity in the SLUA, a conservative 100%CT is assumed.

Typically, when monitoring data are used, the RDF consists of the full distribution of found residues, ½LODs (half the analytical method limit of detection), and zeros. A residue value of zero (0.0 ppm) is assigned to the percentage of the crop that is assumed to be untreated. A residue value of 1/2LOD is assigned to the percentage of the crop that is assumed to be treated, but which did not result in residues above the LOD. The remainder of the RDF consists of actual detected residues, if any. When 100%CT is assumed for a commodity, no zeros are included in the RDF.

When monitoring data for a "not blended" (NB) or "partially blended" (PB) commodity are used for (translated to) a blended (B) commodity, a point estimate is used rather than an RDF. The average monitoring value for the NB or PB commodity is determined and that value is entered as a single point estimate for the blended commodity.

When using field trial data, when a commodity is blended the average field trial value is used along with the maximum %CT estimate; an RDF is not prepared. For NB and PB commodities, a RDF is prepared as for monitoring data and it is based on the maximum %CT estimate.

For some commodities, the raw agricultural commodities (RACs) are processed into other food forms (e.g., apple juice from apples, or refined oil from cottonseed) which may result in a reduction or concentration of the pesticide residues found on the RAC. Concentration or reduction may also occur as foods are prepared for consumption (e.g., peeled, washed, or cooked). In order to account for concentration or reduction of residues in an assessment, two types of processing factors may be used: empirical factors determined in processing studies and model (i.e., DEEM™) default processing factors. For chlorpyrifos, empirical processing factors were available for many food forms and were applied in the dietary assessment where appropriate (peeling, washing, cooking, canning, and juicing). Default processing factors were used for foods for which the empirical processing factors are not appropriate.

The dietary food exposure assessment for the chlorpyrifos 2014 HHRA included the following assumptions:

- All 47 plant-based human food commodities with tolerances in 40 CFR.342, and their associated food forms, were included in the dietary assessment. These include a large variety of the following: root vegetables, Brassica leafy vegetables, legume vegetables, fruiting vegetables, cucurbits, citrus fruit, pome fruit, stone fruit, nuts, grains, and other miscellaneous foods such as banana, grape, strawberry, cranberry, kiwifruit, asparagus, sunflower, fig, and mint.

- All 20 livestock-based human food commodities with tolerances in 40 CFR.342, and their associated food forms, were included in the dietary assessment. These include meat, fat, meat byproducts, milk, and eggs.

- Tolerance level residues were used for sugar beet, kiwifruit, peppermint and spearmint. Field trial residue data for bean (dry), cotton, sunflower, and peanut were used to determine anticipated residues in those food forms. PDP food monitoring data were used to determine residues in all other foods.

- Processing (residue concentration or reduction) factors were used for several foods, where appropriate. A peeling factor was used for sweet potato, peeled apples and pears, and kiwifruit. A cooking and/or canning factor was applied for cooked leafy vegetables and to cooked pumpkin, cherry, peach, and asparagus. Most fruit juices included a processing factor as did dried fruit and fruit peel.

- The maximum %CT was incorporated into the anticipated residue calculations. Maximum %CT estimates ranged from 2.5% (when reported as <2.5% CT) to 100% (when there was no reported %CT value).

- Residues were input as distribution files (RDFs) for the majority of foods, resulting in a

probabilistic assessment. Blended foods and foods for which residues were based on tolerance levels used a single point estimate of residue rather than an RDF, resulting in a deterministic assessment for those foods.

9.2.3 Food Exposure Estimates

The results at the upper percentiles of exposure (99th, 99.5th, and 99.9th) of the chlorpyrifos acute and steady-state food exposure assessments for infants and females of childbearing age are presented in Table 7. Results of Acute and Steady State Dietary (Food Only) Exposure Analysis for Chlorpyrifos below.

Table 7. Results of Acute and Steady State Dietary (Food Only) Exposure Analysis for Chlorpyrifos

Population Subgroup	Acute Exposure (µg/kg/day)			Steady State Exposure (µg/kg/day)		
	95th Percentile	99th Percentile	99.9th Percentile	95th Percentile	99th Percentile	99.9th Percentile)
Infants (<1 year old)	0.050	0.088	0.273	0.044	0.083	0.186
Females (13–49 years old)	0.021	0.041	0.150	0.018	0.029	0.075

The PBPK model was used to generate time course of chlorpyrifos concentrations in blood based on a dietary exposure scenario in which a female adult weighing 72.9 kg following a single daily exposure to chlorpyrifos in food for 42 days. The PBPK simulation results at the 10th to the 99.9th percentile of exposure for the steady state food assessment for females of childbearing age are presented in Table 8.

Table 8. Predicted levels of food exposure to chlorpyrifos from the 2014 revised risk assessment and associated maximum and 24-hour blood concentrations in adult females
(Note: Information here is the same as reported in Table 2).

Percentile of Exposure	Exposure from Calendex	Max Blood Levels of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex	10-hour Blood Levels Post Exposure Values of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex	24-hour Blood Levels Post Exposure Values of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex
10	0.003 µg/kg/day	0.29	0.060	0.021
30	0.005 µg/kg/day	0.48	0.099	0.034
50	0.007 µg/kg/day	0.67	0.139	0.048
70	0.009 µg/kg/day	0.86	0.179	0.062
90	0.014 µg/kg/day	1.33	0.278	0.096
95	0.018 µg/kg/day	1.71	0.358	0.124
97.5	0.023 µg/kg/day	2.19	0.457	0.158

Percentile of Exposure	Exposure from Calendex	Max Blood Levels of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex	10-hour Blood Levels Post Exposure Values of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex	24-hour Blood Levels Post Exposure Values of CPFOS (pg/g) from Food Runs at Various Percentiles of Exposure from Calendex
99	0.029 µg/kg/day	2.76	0.576	0.200
99.5	0.037 µg/kg/day	3.52	0.735	0.255
99.9	0.075 µg/kg/day	7.14	1.490	0.517

9.3 Drinking Water Exposure

Based on the current use profile for chlorpyrifos, the primary and dominate route of drinking water exposure to this pesticide in drinking water is sourced surface water.[20,21] Therefore, the focus of this section is on chlorpyrifos exposure concentrations in surface water. Surface sourced drinking water is processed at local community water treatment facilities and is not nationally distributed. One exception is bottled water. Thus, estimated drinking water concentrations (EDWCs) are intended to capture exposure levels that an individual may ingest on a watershed basis.

The drinking water assessment methods described in this section have undergone previous FIFRA SAP review in the years 1998[22], 1999[23], 2000[24], 2010[25] and 2011[26], and are not subject to the current SAP review but are presented for purposes of assessing the new approach to integrate the exposure assessment with the PBPK model. These methods include use of exposure modeling as well as evaluation of available water monitoring data to fully characterize the potential drinking water exposure.

---

[20] U.S. Environmental Protection Agency (Bohaty, R.), Revised Chlorpyrifos Preliminary Registration Review Drinking Water Assessment, June 20, 2011, PC Code: 059101; DP Barcode: 368388, 389480

[21] U.S. Environmental Protection Agency (Bohaty, R.), Chlorpyrifos: Updated Drinking Water Assessment for Registration Review, December 23, 2014, PC Code: 059101; DP Barcode: 424487

[22] Proposed Methods for Basin-scale Estimation of Pesticide Concentrations in Flowing Water and Reservoirs for Tolerance Reassessment, FIFRA Scientific Advisory Panel Meeting, July 29–30, 1998

[23] Office of Pesticide Programs Policy for the Use of the FQPA 10x Safety Factor; Statistical Methods for Use of Composite Data in Acute Dietary Exposure Assessment; Use of Watershed-derived Percent Crop Areas as Refinement Tool in FQPA Drinking Water Exposure Assessments for Tolerance Reassessment, FIFRA Scientific Advisory Panel Meeting May 25–27, 1999

[24] Assessment of pesticide concentrations in drinking water and water treatment effects on pesticide removal and transformation, FIFRA Scientific Advisory Panel Meeting September 26–29, 2000

[25] Re-evaluation of the Human Health Effects of Atrazine: Review of Experimental Animal and In-vitro Studies and Drinking Water Monitoring Frequency, FIFRA Scientific Advisory Panel Meeting, April 26–29, 2010

[26] Progress Report on Estimating Pesticide Concentrations in Drinking Water and Assessing Water Treatment Effects on Pesticide Removal and Transformation: A Consultation. FIFRA Scientific Advisory Panel Meeting, Sept 29, 2000; SAP Report No. 2001-02. February 12, 2011.

9.3.1 Exposure Modeling

Currently, EPA-OPP estimates pesticide exposure using computer models[27] that simulate pesticide sorption to soil, in-field decay, and runoff from an agricultural field/drainage area following pesticide application(s) as well as resulting concentrations in an adjacent surface water body known as the "index reservoir."[28] Simulations account for a range of different soil, weather, hydrologic conditions, and management/crop use conditions. In addition, all available chemical specific environmental data are considered as well as different labeled application rates. For drinking water assessments, the estimated 1-in-10 year return frequency concentrations from surface water modeling, for either single-day (24-hour concentration for estimating acute exposures) or time-averaged periods (for estimating chronic exposures) is compared to relevant toxicity endpoints of concern.

To date, two drinking water assessments, which include a number of different exposure scenarios, have been completed for chlorpyrifos as part of the registration review process. To illustrate a range of upper bound EDWCs on a national basis, two maximum label rate application scenarios were selected to represent high and low end exposures, *i.e.*, Michigan tart cherries at 5 applications totaling 14.5 pounds per acre per year, and Georgia bulb onions at a single application of 1 pound per acre per year, respectively. The daily (24-hour average) EDWCs for the entire simulation (referenced as time series data) for chlorpyrifos is provided in Figure 9 for the Georgia bulb onion. This simulation represents a pre-plant soil application occurring on December 4 every year for approximately 30 years. Multiple years of meteorological data are simulated to account for the variation in weather that exists over time (*i.e.*, some years are wetter/drier, warmer/cooler than others). This simulation also takes into account required aquatic spray drift buffers for ground applications of 25 feet. Figure 9 shows a peak chlorpyrifos concentration of 8 µg/L (data circled in the figure). The various peak concentrations observed in the time series data represent the various runoff events that occur either as a result of a rainfall event or irrigation. The higher peak concentrations correspond to high rainfall events that follow shortly after application leaving little time for chlorpyrifos to degrade on the application site.

[27] http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#aquatic.
[28] See "Development and Use of the Index Reservoir in Drinking Water Exposure Assessments" at http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/development-and-use-index-reservoir-drinking-water

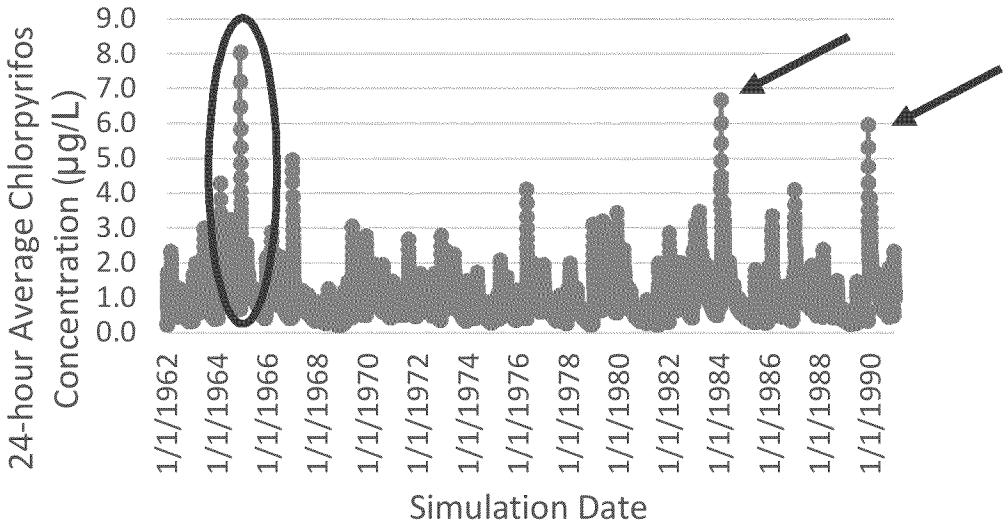


Figure 9. Simulation Time Series Data for Chlorpyrifos Use on Bulb Onion (1 lb a.i./A once per year) in Georgia

As described elsewhere, the 1-in-10 year return frequency concentration is normally used in dietary exposure assessments. For a 30-year simulation, this value corresponds to the third highest peak exposure concentration (arrows indicate the second and third highest concentrations), and in this case, it is approximately 6 µg/L for the 24-hour concentration.

9.3.2 Monitoring Data

The low quantity and/or quality of available monitoring data for many pesticides, including chlorpyrifos and chlorpyrifos-oxon, presents a serious challenge in forecasting drinking water exposure concentrations under various use conditions and geographic scales (national vs. watershed). In general, most available monitoring data are not coordinated with a particular pesticide application, sampling frequencies are typically insufficient to capture durations of exposure concern (*e.g.*, peak), and sampling is usually temporally and spatially limited.[29] It is important to accurately capture peak concentrations to determine upper bound exposure for acute exposure estimates, as well as to best estimate longer term average exposure concentrations (*e.g.*, 21-day average). For these reasons, monitoring data likely do not capture upper bound estimates of exposure for individuals on a national basis. However, a high quality monitoring data set can be informative as it helps elucidate what is happening under current use practices and specific conditions at a given location. For example, Dow AgroSciences (MRID 44711601)[30] submitted daily monitoring data for chlorpyrifos that provides information on the variation in chlorpyrifos

[29] Re-evaluation of the Human Health Effects of Atrazine: Review of Experimental Animal and In-vitro Studies and Drinking Water Monitoring Frequency, FIFRA Scientific Advisory Panel Meeting, April 26-29, 2010

[30] Poletika, N.; Robb, C. (1998) A Monitoring Study to Characterize Chlorpyrifos Concentration Patterns and Ecological Risk in an Agriculturally Dominated Tributary of San Joaquin River: Lab Project Number: ENV96055. Unpublished study prepared by Dow AgroSciences and Paragon Research.

concentrations in time and in space with known chlorpyrifos applications. Sampling was conducted for one year (May 1, 1996 to April 30, 1997) at three locations on the lower reach of Orestimba Creek, a tributary of the San Joaquin River in California. This study includes use information, percent chlorpyrifos use area, and agronomic practices such as irrigation. In addition, use information for chlorpyrifos is available for this watershed in the California Pesticide Use Reporting (PUR) database.[31] Daily time-proportional composite samples were collected, along with weekly grab samples, and the results are presented in Figure 10.

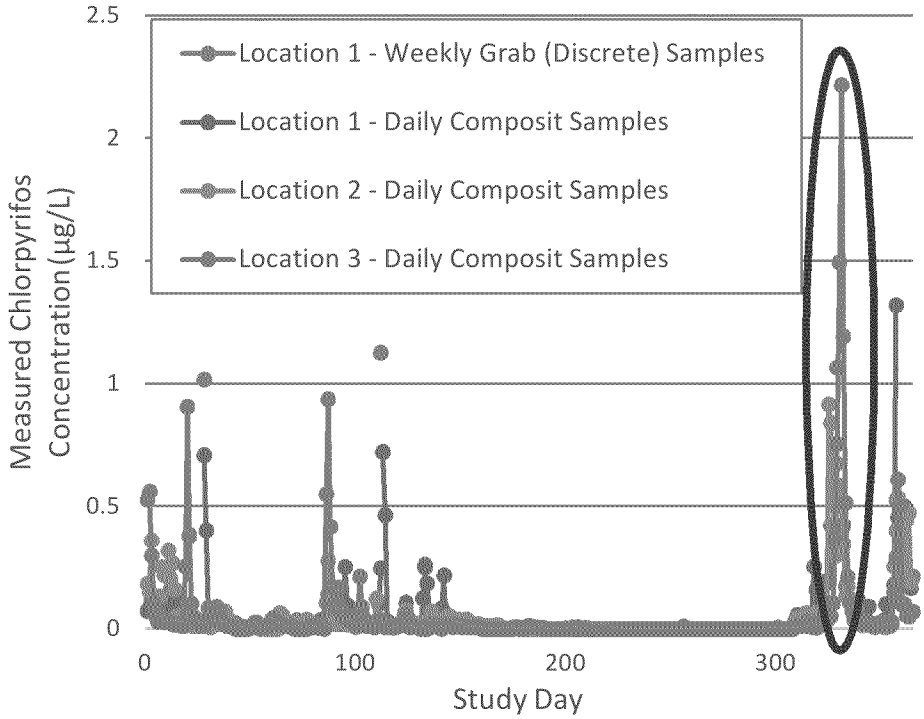


Figure 10. Orestimba Creek Water Monitoring Data (May 1, 1996 to April 30, 1997)

In several cases, the weekly grab samples were observed to have higher concentrations of chlorpyrifos. This finding suggests that the composite sampling methodology used in the study for daily samples resulted in the dilution of peak daily concentrations. The highest measured daily concentration was 2.2 µg/L and was associated with a chlorpyrifos application to alfalfa (0.5 lb a.i./A applied aerially to a 50–70 acre field), followed by flood irrigation. The limit of detection reported for chlorpyrifos in this study is 0.01 µg/L in water.

While Orestimba Creek does not have a drinking water intake, this study is one of the few available daily monitoring data sources with known applications of chlorpyrifos. In addition, the San Joaquin River, for which the Orestimba Creek is a tributary, provides source drinking

[31] California Department of Pesticide Regulation. 2013. Pesticide Use Reporting. Retrieved from CDPR website: http://www.cdpr.ca.gov/docs/pur/purmain.htm

water[32], and chlorpyrifos concentrations observed in this study are not outside the range of those observed in other datasets. Thus, it is reasonable to assume that the magnitude of concentrations observed in this study occur in other locations across the country where drinking water intakes are located.

9.3.3 Drinking Water Treatment

Because drinking water for a large percentage of the population is derived from community water systems that treat raw water[33] prior to consumption, the impact of water treatment on pesticide removal and transformation are considered, when possible, in estimating drinking water exposure.[34,35,36] There are a wide range of drinking water treatment processes utilized by community drinking water systems across the country, including coagulation/flocculation, sedimentation, filtration, and disinfection.[37] The effect of various treatment processes have been investigated for a number of pesticides including chlorpyrifos. The results of one study shown in Table 9. [38] Table 9 suggests that removal of chlorpyrifos is highly dependent on the treatment method employed by an individual community drinking water treatment facility. In the presence of free chlorine, the most common disinfection process utilized by community water systems, chlorpyrifos reduction is high (>90%). However, reduction of chlorpyrifos in the presence of monochloramines, often used as an alternative to chlorine to reduce disinfectant/disinfection by-products, is low (<10%). These results are consistent across multiple publications.[39,40,41]

---

[32] http://www.americanrivers.org/endangered -rivers/2014-report/san-joaquin/

[33] United States Environmental Protection Agency. 1989. Technologies for Upgrading Existing or Designing New Drinking Water Treatment Facilities. EPA/625/4-89/023.

[34] Assessment of pesticide concentrations in drinking water and water treatment effects on pesticide removal and transformation, FIFRA Scientific Advisory Panel Meeting September 26-29, 2000

[35] Progress Report on Estimating Pesticide Concentrations in Drinking Water and Assessing Water Treatment Effects on Pesticide Removal and Transformation: A Consultation. FIFRA Scientific Advisory Panel Meeting, Sept 29, 2000; SAP Report No. 2001-02. February 12, 2011.

[36] U.S. Environmental Protection Agency, Office of Pesticide Programs. The Incorporation of Water Treatment Effects on Pesticide Removal and Transformations in Food Quality Protection Act (FQPA) Drinking Water Assessment, October 25, 2001

[37] U.S. EPA Office of Water 2006 Community Water System Survey, May 2009 available at EPA-815-R-09-002. Retrieved from USEPA website: http://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=2000JTKL.txt.

[38] Chamberlain, E. Shi, H., Wang, T., Ma, Y., Fulmer, A., Adams. C. J Agric. Food Chem. 2012 *60*, 354-363

[39] Duirk, S. W. Desetto, L. M., Davis, G. M., Lindell, C., Cornelison, C. T. Water Research 2010, 44, 761-768

[40] Ormad, M. P., Miguel, N., Matesanz, J. M., Ovelleiro, J. L., Chemosphere, 2008, 71 97-106

[41] Tierney, D. P.; Christensen, B. R.; Culpepper, V. C. Chlorine Degradation of Six Organophosphate Insecticides and Four Oxons in Drinking Water Matrix. *Submitted by Syngenta Crop Protection, Inc.* 2001

Table 9. Chlorpyrifos Reduction under Typical Drinking Water Treatment Conditions; Drinking Water Treatment Processes Utilized by Community Water System Based on Population Served

Treatment Method[a]	FC		MCA		ClO_2		MnO_4-		UV		H_2O_2		O_3		Softening
	pH 6.6	pH 8.6	pH 6.6	pH 8.6	pH 6.6	pH 8.6	pH 6.6	pH 8.6	pH 6.6	pH 8.6	pH 6.6	pH 8.6	pH 6.6	pH 8.6	pH 12
Percent Reduction[b]	90.3	85.7	8.7	9.2	34.3	27.5	15.3	5.2	14.5	1.9	7.6	3.1	60.9	30.3	100.0
System Population Category	Percentage of Plants Performing Each Treatment Practice for Surface Water[c]														
100 or less	98.4		0		0		1.6		3.1		-		-		0
101-500	79		1.2		0		9.2		1.7		-		-		2.5
501-3,300	97.4		2.2		0		7.8		2.2		-		-		3.4
3,301-10,000	80.8		13.7		11		24.7		1.4		-		-		19.2
10,001-50,000	80.5		14.8		8.7		32.9		1.3		-		-		16.9
50,001-100,000	75.1		17.1		18.5		26.8		2.6		-		-		5.2
100,001-500,000	78.9		32.4		14		26.3		4.7		-		-		11.8
Over-500,000	78.0		35.6		2.5		21.2		1.7		-		-		21.2

a. Experimental time was representative of typical drinking water treatment condition
b. See footnote 37 (Chamberlain, 2012)
c. See footnote 36 (EPA community water system survey)

Chlorine (FC); Chlorine dioxide (ClO_2); Chloramines (MCA); Lime/soda ash softener (assumed to be similar to hydrolysis at pH 12); Ultraviolet light (UV); Ozone (O_3); Potassium permanganate (MnO_4-)

Most studies in the open literature do not examine the formation of transformation products under the same drinking water treatment processes. Although, information on water treatment effects on chlorpyrifos is generally limited, there are a few available studies that investigate the transformation of chlorpyrifos in the presence of chlorine.[38,40,42] These studies show that chlorpyrifos converts to chlorpyrifos-oxon. The transformation of chlorpyrifos [phosphothionate group (P=S)] to chlorpyrifos-oxon [phosphorus-oxygen double bond (P=O)] in the presence of chlorine proceeds via rapid oxidation by the oxychlorine species (see Figure 11).

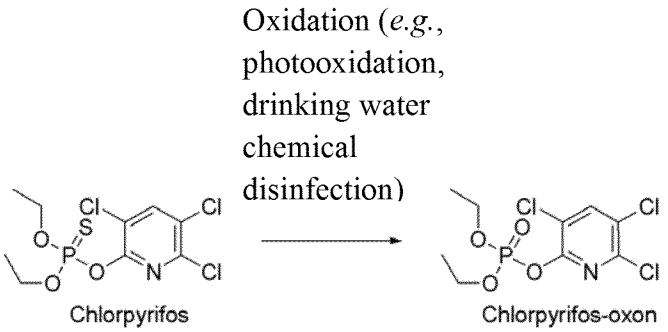


Figure 11. Transformation of Chlorpyrifos to Chlopyrifos-oxon

Once formed as a disinfection by-product, chlorpyrifos-oxon is expected to be relatively stable to drinking water distribution conditions and times (few hours to a few days). No data on physical removal processes such as coagulation/ flocculation, sedimentation, and filtration are available for chlorpyrifos or chlorpyrifos-oxon. However, such processes, with the exception of granular activated carbon[43], have been shown to be ineffective for select organic pesticides.[35] Based on the physical-chemical properties of chlorpyrifos and chlorpyrifos-oxon, granular activated carbon likely reduces the amount of both chemicals to some extent. However, data are not available on the exact removal efficiency for these chemicals treated with granular activated carbon or powder activated carbon. It should be noted that granular activated carbon is not a common treatment practice for all treatment facilities. Additional, powered activated carbon, which is used for taste and odor control, is not used year round.

Limited monitoring data are available for chlorpyrifos and chlorpyrifos-oxon following drinking water treatment. Available sources include the USEPA/USGS Pilot Reservoir Monitoring Program and the USDA Pesticide Data Program. Chlorpyrifos and chlorpyrifos-oxon were not detected in finished water in either of these programs; however, when raw and finished water samples were collected, the correlation between samples could not be made for chlorpyrifos. Thus, the impact of treatment could not be conclusively determined. Nevertheless,

---

[42] Wu, J.; Laird, D. A. Abiotic Transformation of Chlorpyrifos to Chlorpyrifos Oxon in Chlorinated Water. Environ. Toxcol. Chem., 2003, 22(2), 261-264

[43] U.S. Environmental Protection Agency. 1998. Small System Compliance Technology List for the Non-Microbial Contaminants Regulated Before 1996. EPA 815-R-98-002.

USEPA/USGS Pilot Reservoir Monitoring Program for other organophosphates (*e.g.*, malathion) suggests that in the presence of chlorine treatment processes, oxon formation occurs.

In summary, given the wide range of removal and transformation efficiencies of chlorpyrifos under the various drinking water treatment processes, it is assumed that it is possible, depending on the community water system and associated treatment processes, that source water containing chlorpyrifos may result in exposure to chlorpyrifos and chlorpyrifos-oxon in varying amounts. Therefore, in order to address the multitude of water treatment possibilities, a bounding approach was used. That is, to represent those facilities that use disinfectant processes other than free chlorine, 100 percent of the chlorpyrifos entering the facility was assumed to be unchanged in the finished drinking water. In addition, to represent those facilities that employ chlorine as a disinfectant, 100 percent of the chlorpyrifos entering the facility was assumed to convert to chlorpyrifos-oxon (not presented here). The treatment methods and data for the associated population served data are provided in Table 9. In general, chlorine is used by treatment facilities that serve small populations, while treatment facilities serving larger populations tend to use alternative disinfection processes such as monochloramine more often. Chlorpyrifos and chlorpyrifos-oxon are not expected to degrade during distribution, as distribution times typically range from a few hours to a few days. As such, these chemicals are considered residues of exposure concern in drinking water.

Because the CCCEH researchers measured parent chlorpyrifos in blood as the metric of internal exposure and the majority of animal toxicity studies are conducted with the parent compound, the most robust information related to neurodevelopmental effects is associated with chlorpyrifos. As such, this proposed approach focuses on exposure to chlorpyrifos instead of chlorprifos-oxon. In short, the agency is not pursuing the use of the oxon as the moiety of concern for the neurodevelopmental outcomes because there are limited data to validate the PBPK model estimates of chlorpyrifos-oxon. As such, EPA has more confidence in the PBPK model estimates of chlorpyrifos blood levels compared to chlorpyrifos-oxon.

9.3.4. Anticipated Exposure Scenarios and PBPK Model Simulations

To investigate the potential chlorpyrifos exposure to people in other areas of the country that may be exposed to chlorpyrifos via drinking water, the cord blood levels of chlorpyrifos observed in the CCCEH study are compared with PBPK simulated chlorpyrifos blood concentrations following consumption of drinking water containing chlorpyrifos for infants and females of childbearing age.

9.3.4.1 Females of Childbearing Age (13-49 years old)

Adult females may be exposed to chlorpyrifos as a result of consuming drinking water that contains chlorpyrifos. The PBPK model was used to predict the time course of chlorpyrifos concentrations in venous blood for a female adult weighing 72.9 kg (160.7 lbs.) following

exposure to chlorpyrifos in drinking water based on model EDWCs (Figure 9) and measured concentrations (Figure 10).

A subset (120 days) of continuous daily concentrations from time series data (Georgia Bulb Onion low-exposure scenario) was used as an input into the PBPK model. The 120-day time series used in the PBPK model simulation contained the day with the highest daily average concentration of chlorpyrifos (Figure 9). The entire time series was not used because running simulations for a longer exposure duration exceeded the runtime limit of the software, acslX (Aegis Technologies Lab, Inc., Huntington, AL). The simulated adult female is assumed to consume drinking water four times a day, with a daily consumption volume of 1.71062 L (57.8 oz.). The results of the PBPK simulation for this low end exposure scenario are provided in Figure 12. The peak chlorpyrifos blood concentration is approximately 6.99 pg/g. Chlorpyrifos blood concentrations are greater than 1 pg/g for most of the 120-day simulation.

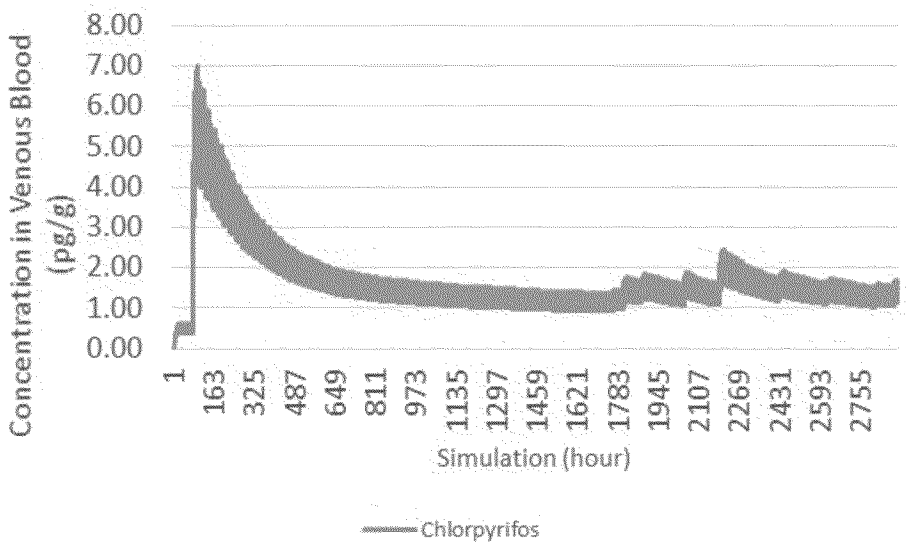


Figure 12. PBPK Estimated Chlorpyrifos Venous Blood Concentrations for Adult Female Based on 120-day Time Series Data for Chlorpyrifos Use on Bulb Onion (1 lb a.i./A once per year) in Georgia

Due to the discreet exposure scenarios that occur for the food and worker exposures, the agency is using the 10 and 24 hours post-exposure to characterize the asymptotic portion of the PK profile. In the case of drinking water exposure, exposures may occur at multiple times throughout the day when drinking and vary widely across a year. As such, discreet exposure periods are more difficult to define. During time periods of higher chlorpyrifos concentrations in drinking water, the PK profile does not transfer from the absorption/distribution phase of PK into the terminal ½ life phase. As such, the agency can only compare the lowest point within a range of exposure days.

Since the chlorpyrifos venous blood concentration is linearly related to the chlorpyrifos concentration in water within the range of these concentrations, none of the concentrations

excluded from the current simulation, due to the software limitations described above, should exceed the maximum blood concentration predicted using the times series containing the maximum daily average water concentration. While this time series contains the highest daily concentration, there are two other time periods that have similar peak 24-hour average concentrations (noted with arrows in Figure 9), thus when entered directly into the PBPK model would be expected to result in similar levels of chlorpyrifos in the blood. The lowest peak 24-hour daily concentration (approximately 6 µg/L) from the three peak exposure periods corresponds to the 1-in-10 year return frequency concentration that is typically reported in a drinking water assessment. Moreover, this time series also represents the lower end of exposures expected for chlorpyrifos based on current maximum label rates for chlorpyrifos.

The PBPK model was also used to predict the time course of chlorpyrifos concentrations in venous blood for an adult female based on exposure to daily measured chlorpyrifos concentrations from Orestimba Creek (Figure 10). As described previously, daily concentrations were entered into the PBPK model. The limit of detection for chlorpyrifos in this study of 0.01 µg/L was used when no detection was reported as the actual concentration could range between zero and the detection limit. The PBPK model was used to predict the time course of chlorpyrifos concentrations in venous blood for an adult female with body weight of 72.9 kg (160.7 lbs.) following exposure to chlorpyrifos in drinking water based on 120 days of measured chlorpyrifos concentrations from the Orestimba Creek monitoring data. The simulated adult female is assumed to consume drinking water four times a day, with a daily consumption volume of 1.71062 L (57.8 oz.). The concentration from the 120 days corresponding to the peak measured concentration were extracted for use (data are circled in Figure 10). Results are shown in Figure 13. The peak chlorpyrifos blood concentration is approximately 1.97 pg/g.

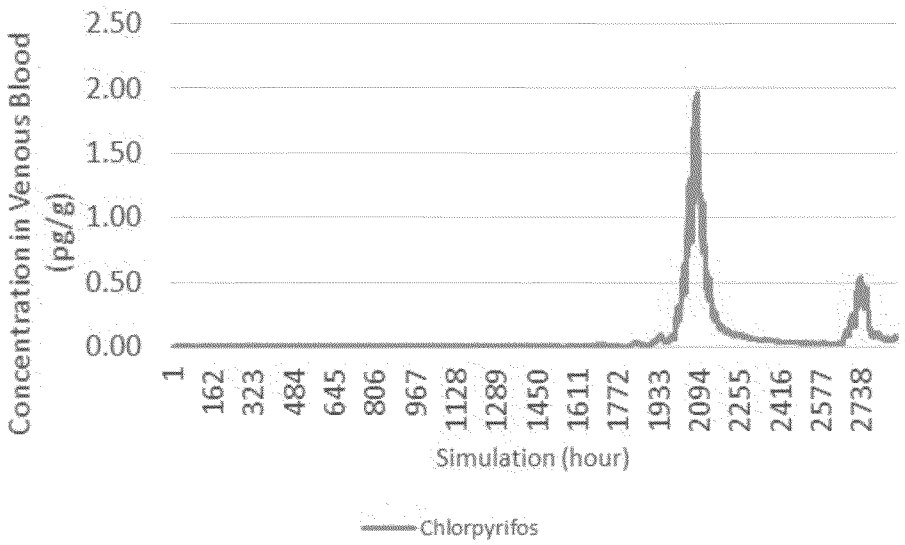


Figure 13. PBPK Estimated Chlorpyrifos Venous Blood Concentrations for Adult Female Based on 120-day Time Series Data for Chlorpyrifos Monitoring Data for Orestimba Creek

9.3.4.2 Infants: Formula Fed (with water)

A formula-fed infant may be exposed to chlorpyrifos as a result of the use of tap water to mix formula. The PBPK model was used to predict the time course of chlorpyrifos concentrations in venous blood for an infant with a fixed body weight of 4.8 kg (10.6 lbs.) following exposure to chlorpyrifos in drinking water based on model EDWCs (Figure 9) and measured concentrations (Figure 10) of chlorpyrifos. This was done by taking a subset (120 days) of continuous daily concentrations of chlorpyrifos from an example time series (Georgia Bulb Onion low-exposure scenario) as input into the PBPK model. The simulated infant is assumed to consume drinking water six times a day, with a daily consumption volume of 0.688557 L (23.3 oz.). The peak chlorpyrifos venous blood concentration is 21.6 pg/g (Figure 14). Chlorpyrifos blood concentrations are greater than 5 pg/g for most of the 120-day simulation.

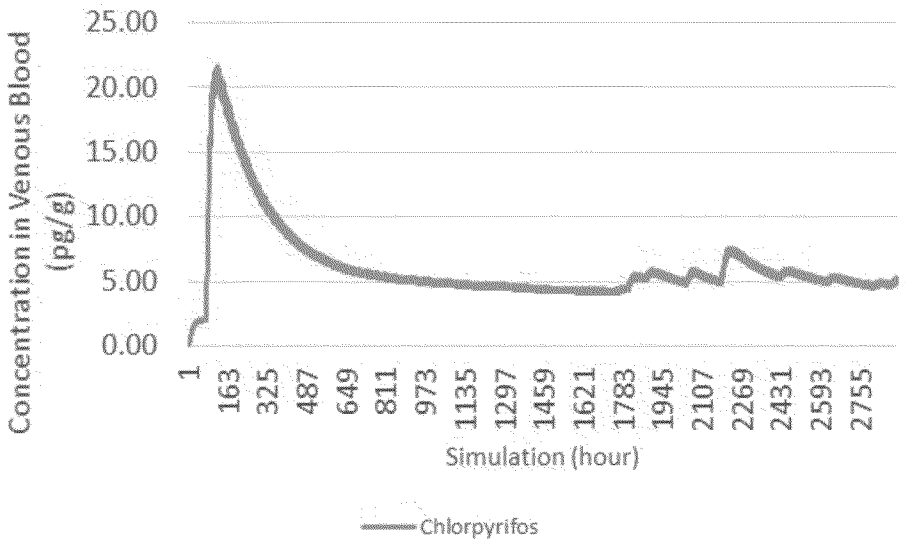


Figure 14. PBPK Estimated Chlorpyrifos Venous Blood Concentrations for Infants Based on 120-day Time Series Data for Chlorpyrifos Use on Bulb Onion (1 lb. a.i./A once per year) in Georgia

As noted above, during periods of higher chlorpyrifos concentrations in drinking water, the PK profile does not transfer from the absorption/distribution phase of PK into the terminal ½ life phase. This is particularly true for infants who consume bottles at regular, frequent intervals throughout the day and night.

The PBPK model was also used to predict the time course of chlorpyrifos concentrations in venous blood for infants based on exposure to daily measured chlorpyrifos concentrations from Orestimba Creek (Figure 10). As described previously, daily concentrations were entered into the PBPK model. In this case, the PBPK model was used to predict the time course of chlorpyrifos concentrations in blood for infants with a fixed body weight of 4.8 kg (10.6 lbs.) following exposure to chlorpyrifos in drinking water based on 120 days of measured chlorpyrifos concentrations from the Orestimba Creek monitoring data. The 120 days corresponding to the

peak observed concentration were extracted for use. The results of the PBPK simulation for this low end exposure scenario is provided in Figure 15. The peak chlorpyrifos blood concentration is approximately 6.24 pg/g.

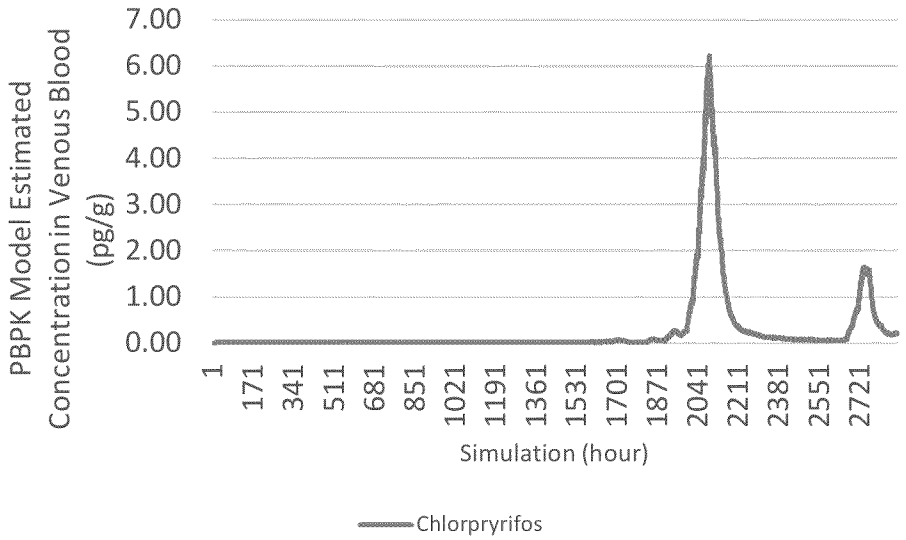


Figure 15. PBPK Estimated Chlorpyrifos Venous Concentrations for Infants Based on Time Series Data for Chlorpyrifos Monitoring Data for Orestimba Creek

9.3.5. Drinking Water Summary

Given the wide range of drinking water treatment methods used across the United States, an individual may be exposed to both chlorpyrifos and chlorpyrifos-oxon to varying degrees via drinking water. This analysis focused on exposure to chlorpyrifos via drinking water and the associated PBPK estimated cord blood chlorpyrifos concentrations. This was done using two different drinking water exposure scenarios for infants and females of childbearing age. A 120-day time series for a national low-end exposure scenario (*i.e.*, Georgia Onion) and measured concentrations from a daily monitoring program conducted for Orestimba Creek in California were input into the PBPK model. The corresponding chlorpyrifos concentrations in blood were presented and are summarized in Table 10. Other use scenarios are expected to result in higher estimated drinking water concentrations resulting in higher blood concentrations.

Table 10. Summary of PBPK Model Estimated Maximum Chlorpyrifos Blood Concentrations Following Two Different Drinking Water Exposure Scenarios

Population	Exposure Scenario	
	Model Estimated	Measured
Infants: Formula Fed (with water)	21.6 pg/g	6.24 pg/g
Females of Childbearing Age (13–49 years old)	6.99 pg/g	1.97 pg/g

9.4 Worker Exposure

The agency evaluated all potential occupational exposure scenarios as part of the 2014 HHRA. For this issue paper for review by the FIFRA SAP, internal dose estimates of chlorpyrifos were assessed for selected pesticide handler exposure scenarios which are considered among those with comparatively low occupational exposure potential. These 'low' occupational scenarios are presented for purpose of illustrating how occupational handler exposures and PBPK modeling can be integrated. A brief overview of the data and methods used by the agency to evaluate occupational pesticide handler exposures is provided here for context. These data and methods have undergone extensive peer review and are not subject to the 2016 FIFRA SAP review.[44]

9.4.1 Methods

In the risk assessment process, the agency considers all potential scenarios that could lead to exposures associated with a pesticide's use. As noted above, a comprehensive risk assessment was completed for chlorpyrifos but a select number of scenarios for pesticide handlers have been used to develop illustrative analyses for consideration. As such the methods described below focus on the types of selected exposures. The agency uses the term "handlers" to describe those individuals who are involved in the pesticide application process. There are distinct job functions or tasks related to applications and exposures can vary depending on the specifics of each task. Job requirements (*e.g.*, the amount of chemical used), the kinds of equipment used, the target pest being treated, and the level of protection used by a handler can cause exposure levels to differ in a manner specific to each application event. The following represent handler activities which are assumed to occur by the agency:

- Mixer/Loaders: individuals perform tasks in preparation for an application. For example, prior to application, mixer/loaders would mix a liquid pesticide concentrate with water and load it into the holding tank of the airplane or groundboom equipment;
- Applicators: individuals operate application equipment during the release of a pesticide product onto its target pest;
- Mixer/Loader/Applicators**:** individuals who perform all aspects of the pesticide application process; and,
- Flaggers**:** individuals that guide aerial applicators during the release of a pesticide product onto its target.

The agency uses the term "unit exposure" to describe the exposure metric which serves as the basis for assessing handler exposures to pesticides. Unique unit exposure values have been developed from available data sources for each handler scenario considered and are expressed as mass of pesticide active ingredient exposure per unit mass of active ingredient handled (*e.g.*, µg/lb a.i.). A scenario refers to a specific type of application equipment, formulation type, job function, and level of personal protective equipment (PPE). The agency then uses unit exposures

[44] http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/occupational-pesticide-handler-exposure-data

generically to estimate exposure for all pesticides which may have a particular exposure scenario associated with its use. Additionally, other key pieces of information such as the allowable uses on pesticide labels are also important when defining exposure scenarios and calculating exposure estimates (*e.g.*, application rates on specific crops and allowable types of equipment).

As noted above, the occupational exposure methodologies and exposure data sources used have undergone extensive scientific peer review. Since the 1980s, the agency has required pesticide handler exposure data in order to assess the occupational risks of both new and existing pesticides. The first occupational exposure testing guidelines were established at this time. These guidelines were intended to standardize the methodology used to conduct the studies necessary to allow the agency to determine the potential exposures and consequent risks associated with the activities relating to the occupational use of pesticides. In the early 1990s, the Pesticide Handlers Exposure Database (PHED)[45] was constructed in order to estimate exposures resulting from mixing/loading/applying pesticides. The data assembled for use in this database was taken from published literature as well as from industry studies submitted to the agency. Subsequent to PHED, the Outdoor Residential Exposure Task Force (ORETF) was formed in 1994 to satisfy mixer/loader/applicator exposure data required by the agency relating to professional lawn care operators. Additionally, the Agricultural Handler Exposure Task Force (AHETF) was established in 2001 to jointly address ongoing, product-specific agency exposure data requirements. These databases are used as the main sources for estimating occupational exposures to workers handling pesticides for both registration and reregistration actions. PHED is the basis of exposure data for most pesticide handler exposure assessments; however, given changes in cultural production practices over time and the limitations of the PHED data, it has become more appropriate to make use of the more current, AHETF data, as it becomes available because these data better reflect the use of technologies in present day agriculture. Currently recommended unit exposures for agency occupational handler exposure assessment purposes are available on the EPA website.[46]

The scenario-based approach is consistent with the agency's guidelines for exposure assessment which can be found on the EPA website.[47] In addition, it should also be noted that generation of new exposure data are reviewed by the agency's Human Studies Review Board (HSRB) under most circumstances.[48]

---

[45] http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/occupational-pesticide-handler-exposure-data#phed

[46] http://www2.epa.gov/pesticide-science-and-assessing-pesticide-risks/exposure-surrogate-reference-table

[47] http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=15263

[48] http://www.epa.gov/osa/human-studies-review-board

PBPK Model Usage

The use of the PBPK model is based on the exposure scenario and methods described above. However, specific inputs are required to use the PBPK model for estimation of chlorpyrifos blood levels corresponding to chlorpyrifos occupational exposures.

- Exposure Pattern: An 8-hour workday and a 5-day work week was used which is the standard for this type of exposure analysis. Two days were simulated as non-exposure days to simulate days off from work. Worker exposure scenarios were considered for 2 weeks of occupational exposures followed by a 30-day recovery period.
- Use rates: Typical use rates have been used in this 2016 analysis instead of the maximum application rate allowable for the agricultural use site (crop) from currently registered product labeling. For the 2014 HHRA, maximum application rates were used.
- Route of Exposure: For occupational workers, dermal and inhalation exposures are expected and were calculated as noted above. For PBPK modeling, both dermal and inhalation exposure inputs were input into the model and the results reflect the total of both types of exposures.
- Body weight: A female, with a body weight of 69 kg was used due to concerns for potentially pregnant women and the potential uncertainties regarding neurodevelopmental effects on their children. The agency considers women of child bearing age to be between the ages of 13 to 49 years.
- Dermal surface area exposed: For PBPK modeling of chlorpyrifos exposures, 100% of the dermal surface area was assumed to be exposed from conduct of work activities.
- Hygiene: A daily shower was assumed following each day which means there is no additivity between chlorpyrifos exposure due to sequential days of work.
- Inhalation Conversion: Because of the way exposure data are compiled, the standard agency occupational exposure method estimates daily inhalation exposures on a (mg/day) basis and not as an exposure concentration (mg/L). The coding of the PBPK model requires (mg/day) daily inhalation doses be converted to an air concentration for modeling purposes. The following equation was used to make this calculation:

Airborne concentration (mg/L) = Exposure (mg/day) / [Breathing Rate (L/min) * 60 minutes/hour * 8 hours/day]

Note: a breathing rate of 8.3 L/minute was assumed for applicator work activities and 16.7 L/minute for mixer/loader exposure activities[49].

Occupational Handler Exposure Scenarios

For this evaluation a total of 7 'low' occupational handler exposure scenarios were assessed as follows:

Mixer/Loader Activities

- Mixing/loading liquid (EC) for treatment of corn by groundboom soil incorporation at 0.50 lb a.i./A

[49] NAFTA - Dept. of Pesticide Regulation (DPR), California EPA, HSM-98014, April 1998. http://www.cdpr.ca.gov/docs/whs/memo/hsm98014.pdf

- Mixing/loading liquid (EC) for treatment of cole crops[50] by soil incorporation at 1.0 lb a.i./A
- Mixing/loading dry flowable in water soluble packets (WSP) for treatment of cole crops by groundboom soil incorporation at 1 lb a.i./A

Applicator Activities

- Applying sprays via groundboom soil incorporation to corn at 0.50 lb a.i./A
- Applying sprays via groundboom soil incorporation to cole crops at 1 lb a.i./A

Seed Treatment

- Loading/applying liquid (EC) for seed treatment of beans and peas at 0.00058 lb a.i./lb seed

Seed Planting

- Planting corn seed treated at 0.00058 lb a.i./lb seed

9.4.2 Results

Figures 16 and 17 present the PBPK modeling profile of a single, example 'low' occupational exposure scenario, application via groundboom equipment for cole crops at a rate of 1 lb a.i./A. This example exposure scenario, which is the lowest of all example scenarios evaluated, was selected for purpose of comparison to the internal dose profiles resulting from dietary exposures (*i.e.*, food and drinking water) to chlorpyrifos. It presents the full profile of modeled internal dose estimated for the example occupational scenario. When modeled, all 7 occupational exposure scenarios result in the similar PK profile for chlorpyrifos as has been described previously.

Figure 16 (full profile) below shows the sawtooth pattern as the daily, rapid increase in internal dose during the occupational exposure period followed by a rapid decline immediately after the exposure period ends.

[50] Examples of cole crops include: brussels sprout, cauliflower, kale, broccoli

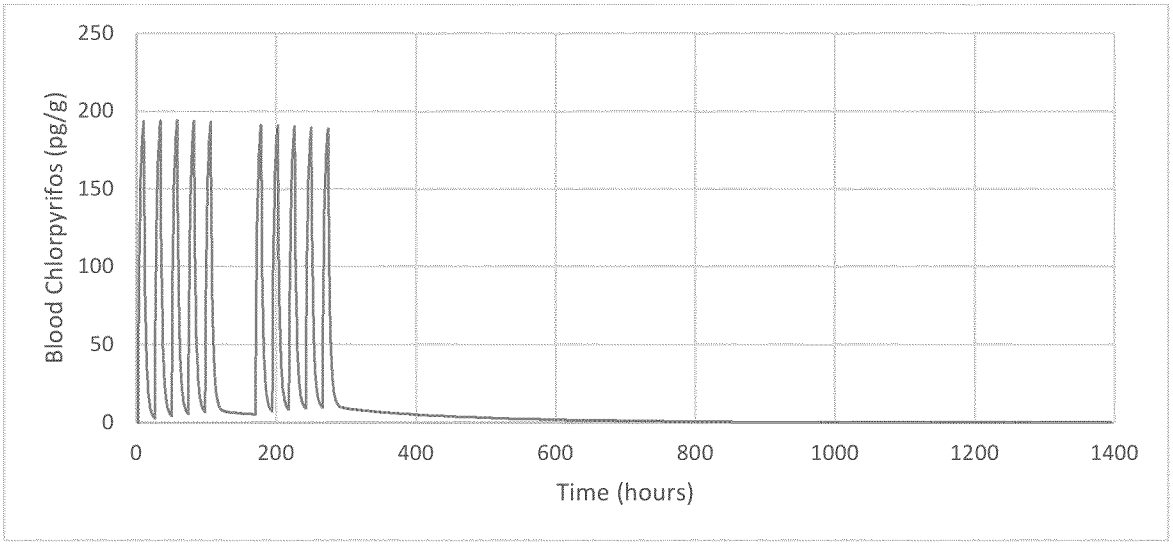


Figure 16. PBPK modeling (full profile) estimation of chlorpyrifos venous blood concentrations (pg/g) per unit time (hours) resulting from the occupational exposure scenario, application via groundboom for cole crops at 1 lb a.i./A.

Figure 17 (sub-set) shows the internal dose just following the end of the modeled occupational exposure (*i.e.*, a period of 2 work weeks). The internal dose curve flattens as terminal half-life is achieved causing an asymptotic appearance.

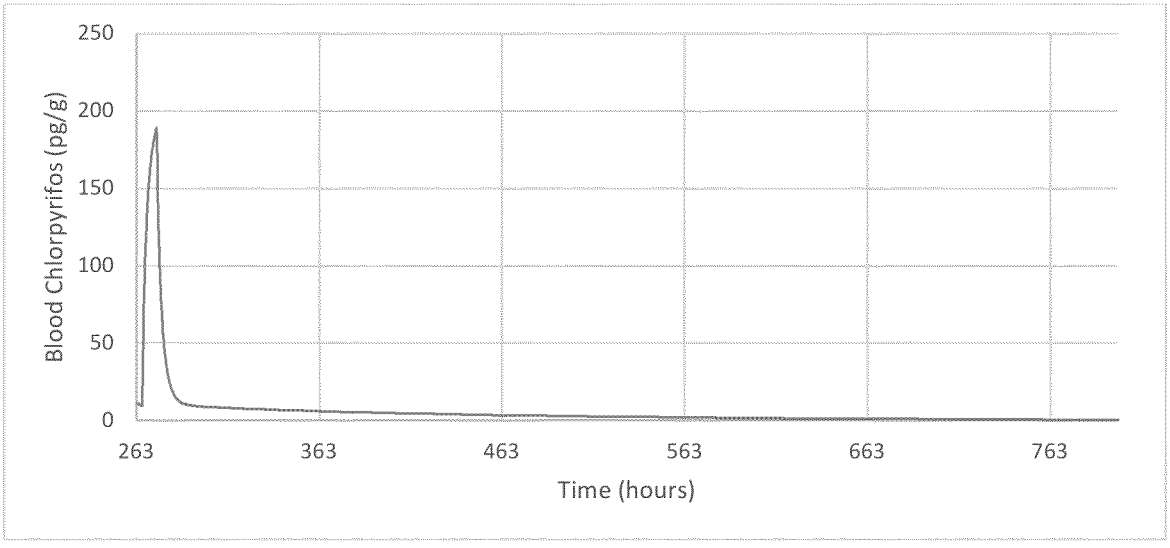


Figure 17. PBPK modeling (sub-set of above Figure 16) of chlorpyrifos blood concentrations (pg/g) per unit time (hours) resulting on the final day from the example occupational exposure, application via groundboom for cole crops at 1 lb a.i./A

Tables 11 and 12 present the results for all 7 example occupational exposure scenarios including dermal and inhalation exposures estimated with use of standard agency methods and inputs, and the modeled maximum, 24-hour venous blood chlorpyrifos concentrations (pg/g), and 10-hour venous blood chlorpyrifos concentrations following the final (12^{th}) day of peak exposure (pg/g) .

Tables 11 and 12 present the results of the mixing/loading and application of chlorpyrifos by groundboom equipment, and the results of seed treatment and planting, respectively. For the mixing/loading and application by groundboom equipment scenarios (Table 11), the maximum modeled venous blood chlorpyrifos levels range from 194 to 413 pg/g, the 24-hour venous blood chlorpyrifos levels range from 3.0 to 8.0 pg/g, and the 10 hour venous blood chlorpyrifos level following the final day of peak exposure range from 16.0 to 38.4 pg/g. For the seed treatment exposure scenario (Table 12), the maximum modeled venous blood chlorpyrifos level is 954 pg/g, the 24-hour venous blood chlorpyrifos level is 12.4 pg/g, and the 10-hour venous blood chlorpyrifos level following the final day of peak exposure is 71.1 pg/g. For the seed planting exposure scenario (Table 12), the maximum modeled venous blood chlorpyrifos level is 214 pg/g, the 24-hour venous blood chlorpyrifos level is 2.8 pg/g, and the 10-hour venous blood chlorpyrifos level following the final day of peak exposure is 16.0 pg/g.

Table 11. Occupational dermal and inhalation exposures used for PBPK modeling of mixer/loader and applicator example low exposure scenarios and the maximum venous blood chlorpyrifos (pg/g) resulting from modeling.

Exposure Scenario				App. Rate	Area Treated	Dermal Unit Expo-sure[1]	Dermal Exposure[2]	Inhalation Unit Exposure[1]	Inhalation Exposure[2]	Airborne Concen-tration[3]	Maximum Venous Blood Chlor-pyrifos	24-Hour Venous Blood Chlor-pyrifos	10 Hours After the Last Peak on Day-12 Blood Chlor-pyrifos
Worker Activity	Form.	Appli-cation Equip-ment/ Type	Use Site	Value (lb a.i./A)	Value (Acres)	Engineering Controls							
						Value (ug/lb a.i.)[1]	Value (mg/day)[2]	Value (ug/lb a.i.)[1]	Value (pg/g)	Value (mg/L)	Value (pg/g)	Value (pg/g)	Value (pg/g)
Mixer/ Loader	EC	Ground-boom/ Soil Incor-poration	Corn (pre-plant)	0.50	200	8.6	0.86	0.083	0.0083	$1.4x10^{-6}$	413	6.6	34.4
			Cole Crops	1.0	80		0.69		0.0066	$8.3x10^{-7}$	329	5.3	27.4
	Dry Flow-able in WSP						0.78		0.019	$2.4x10^{-6}$	404	8.0	38.4
Appli-cator	Spray (all starting forms)		Corn (pre-plant)	0.50	200	5.1	0.51	0.043	0.0043	$1.1x10^{-6}$	243	3.8	20.0
			Cole Crops	1.0	80		0.41		0.0034	$8.6x10^{-7}$	194	3.0	16.0

1. Based on the "Occupational Pesticide Handler Unit Exposure Surrogate Reference Table" (September 2015)
2. Dermal Exposure (mg/day) = Dermal Unit Exposure (µg/lb a.i.) × Conversion Factor (0.001 mg/µg) × Application Rate (lb a.i./acre[1]) × Area Treated (A/day).
3. Inhalation Exposure (mg/day) = Inhalation Unit Exposure (µg/lb a.i.) × Conversion Factor (0.001 mg/µg) × Application Rate (lb a.i./acre) × Area Treated (A/day).
4. Airborne Concn (mg/L) = Exposure (mg/day) / (Breathing Rate (L/min) * 60 min/hr * 8 hours/day)

Table 12. Example model runs for mixing/loading EC for treatment of corn by groundboom soil incorporation at 0.50 lb a.i./A and planting corn seed treated at 0.00058 lb a.i./lb seed.

Exposure Scenario			Application Rate	Area Treated	Dermal Unit Exposure[1]	Dermal Exposure[2]	Inhalation Unit Exposure[1]	Inhalation Exposure[3]	Airborne Concen-tration[4]	Maximum Venous Blood Chlor-pyrifos	24-Hour Venous Blood Chlor-pyrifos	10 Hours After the Last Peak on Day-12 Blood Chlor-pyrifos
Worker Activity	Form.	Use Site	Value (lb a.i./lb seed)	lbs seed treated/ day	Value (ug/lb a.i.)[1]	Value (mg/day)[2]	Value (ug/lb a.i.)[1]	Value (mg/day)[2]	Value (mg/L)	Value (pg/g)	Value (pg/g)	Value (pg/g)
Loader/ Appli-cator	EC	Beans, Peas	0.00058	200,000	18 (DL/G)	2.1 (DL/G)	0.068 (PF5)	0.0079 (PF5)	$1.4x10^{-8}$	954	12.4	71.1
Worker Activity	Form.	Use Site	Value (lb seed/A)	Value (Acres)	Value (ug/lb a.i.)[1]	Value (mg/day)[2]	Value (ug/lb a.i.)[1]	Value (mg/day)[2]	Value (mg/L)	Value (pg/g)	Value (pg/g)	Value (pg/g)
Seed Planting	EC	Corn	15	80	250 (SL/G)	0.47 (DL/G)	0.068 (PF5)	0.0013 (PF5)	$4.6x10^{-9}$	214	2.8	16.0

1. Unit Exposures from HED Exposure Science Advisory Council Policy 14: Standard Operating Procedures for Seed Treatment
2. Dermal Exposure (mg/day) = Dermal Unit Exposure (µg/lb a.i.) × Conversion Factor (0.001 mg/µg) x Application Rate (lb a.i./lb seed) × Amount Planted (lb seed/day)
3. Inhalation Exposure (mg/day) = Inhalation Unit Exposure (µg/lb a.i.) × Conversion Factor (0.001 mg/µg) x Application Rate (lb a.i./lb seed) × Amount Planted (lb seed/day)
4. Airborne Concn (mg/L) = Exposure (mg/day) / (Breathing Rate (L/min) * 60 min/hr * 8 hours/day)

9.4.3 Worker Exposure Summary & Preliminary Conclusions

The PK profile of chlorpyrifos in blood predicted for the 'low' occupational exposure scenarios are consistent with the other chlorpyrifos exposure scenarios modeled, dietary exposures (*i.e.*, food and drinking water) and residential post-application exposures which may have occurred in the CCCEH cohort prior to the voluntary cancellation of indoor products. That is, the profile of chlorpyrifos in the blood rapidly increases during the exposure period and then rapidly decreases following exposure, followed by a terminal half-life phase.

The maximum venous blood concentrations predicted for the example 'low' occupational exposure scenarios are all well in excess of those modeled for dietary exposures. For example, the lowest of the 7 occupational exposure scenarios, application of chlorpyrifos to cole crops via groundboom at an application rate of 1.0 lb a.i./A (presented in Figures 16 and 17), resulted in a predicted maximum blood concentration of chlorpyrifos of 194 pg/g. Even at this low level of occupational exposure, the blood concentration is approximately 27 times greater the maximum blood concentration from food at the 99.9th percentile, 7.1 pg/g (Table 8), and approximately two orders of magnitude greater than the maximum concentration predicted from drinking water based on chlorpyrifos monitoring data for Orestimba Creek (Figure 13). The agency notes that the majority of the occupational exposure scenarios assessed for the 2014 human health risk assessment result in a greater risk potential than those predicted for the 7 'low' occupational exposure scenarios. An additional uncertainty factor is being retained to account for the same concerns that agency had for retaining the FQPA SF for dietary exposures to infants, children, youths and women of childbearing age. Similarly, this database uncertainty factor is retained for adult workers due to the concerns for potentially pregnant women and the uncertainties regarding neurodevelopmental effects. Accordingly, the same proposed internal PoD with a 100X UF/SF will apply for both dietary and occupational exposures.

10.0 Discussion & Next Steps

In 2008 and 2012, the FIFRA SAP cautioned the agency against using the biomonitoring data from epidemiology studies, particularly those from CCCEH, to directly derive PoDs due, in large part, to uncertainties associated with a lack of knowledge about timing of indoor chlorpyrifos application(s) and the single measures of exposure (cord blood) that may not reflect exposure patterns over the course of an entire pregnancy or temporal exposure patterns could have coincided with unknown window(s) of susceptibility. However, the 2012 SAP also recommended that the agency consider use of a PBPK model to further characterize the dose estimates in the epidemiology studies. Since the 2012 FIFRA SAP, epidemiologists from the three US cohorts have continued to publish papers on the adverse effects in children up to ages 7–11 suggesting the lack of reversibility of the outcomes; these findings have heightened the agency's concern. Moreover, the PBPK model has continued to develop and mature to the point of use in regulatory decision making. In light of these developments since the 2012 FIFRA SAP, the agency has followed up on the 2012 SAP recommendation and has conducted additional

characterization of the pharmacokinetic profile of simulated exposures from oral and dermal exposures.

The analysis described here highlights the important context of the cohort and biomonitoring data-namely that when the cord blood were taken the mother would have delivered a baby and labor and delivery is a process that takes hours. These events would have taken sufficient time for the levels of chlorpyrifos in blood to drop substantially and likely plateau to the horizontal asymptote. The agency is explicitly assuming that the biomonitoring data reported by CCCEH were collected at low points on the PK profile, particularly in the terminal clearance phase where such blood concentrations are not changing dramatically.

In this labor and delivery context, the exposure characterization analysis described in Section 6.0 supports the plausibility of the CCCEH reported values across time. Specifically, the blood concentrations (**Table 3**) from the simulated perimeter, carpet scenario closely match the CCCEH cord blood and maternal data for 1998/1999 and 2000 (*i.e.*, *before* the cancellation of indoor uses). Moreover, the blood concentration data in **Table 2** for food exposure across the distribution closely match the CCCEH cord blood and maternal data for 2001–2004 (*i.e.*, *after* the cancellation of indoor uses). As such the agency's exposure characterization analysis compared to the CCCEH data in **Figure 1** support the conclusion that the reported biomonitoring data reflect the likely exposure patterns of chlorpyrifos across the time period of 1998–2004 and thus provide confidence in their use in risk assessment.

Table 13 provides the notable uncertainties listed by the 2012 FIFRA SAP (p. 48 of the report) and provides a cross reference to the section of this document where the uncertainty is addressed by the agency. The CCCEH exposure characterization in Section 6.0 addresses several of the listed uncertainties such as the timing of pesticide applications, limited number of blood sampling times, lack of understanding of critical windows of susceptibility, and plausibility of the exposure levels. Others such as the modest sample size, moderate to large exposure differences need for significance, and generalizability of the cohort to other populations have been addressed in the agency's proposals to retain the 10X intra-species factor and/or the FQPA 10X Safety Factor. As described in Section 7.1, the agency is aware of these uncertainties. However, the agency believes these uncertainties have been sufficiently addressed and do not prevent the transition from using AChE inhibition as the critical effect to neurodevelopmental outcomes for quantitative risk assessment. Moreover, despite the listed uncertainties, there is different uncertainty associated with continuing to use the AChE inhibition effect for deriving the PoDs. Specifically, the internal blood concentrations required to achieve 10% RBC AChE inhibition are substantially higher than those reported by CCCEH and associated with neurodevelopmental outcomes suggesting that the RBC AChE inhibition PoDs are not sufficiently health protective.

Based on human health risks identified in the 2014 human health risk assessment, the agency published a 2015 proposed tolerance revocation for chlorpyrifos. If the revocation action becomes effective as proposed, it would likely lead to the end of chlorpyrifos use on food crops or in processed food produced in or imported into the U.S. The agency is continuing to move forward with the regulatory steps necessary to revoke the chlorpyrifos tolerances.

In summary, this issue paper has described the characterization of predicted exposures to women in the CCCEH prior to giving birth along with a proposed approach for using the CCCEH cord blood data to derive a PoD for use in quantitative risk assessment. In addition, this issue paper proposes an innovative approach to using the PBPK model to integrate external and internal dose from food, water, and occupational exposure assessment for comparison with the proposed internal RfD for dietary exposure and related target MOE for worker/residential exposure.

The agency is soliciting comments from the SAP and the public on the science issues raised in this issue paper.

Table 13. Cross-reference of uncertainties identified by the FIFRA SAP (2012) on using the CCCEH biomonitoring data for deriving a PoD.

Uncertainty Identified by the FIFRA SAP (2012, p. 48)	Section Where Considered	Comment
Relatively modest sample sizes which limited the statistical power to classify some meaningful differences as statistically significant and to examine the effect of modification by race/ethnicity and other characteristics.	8.2. FQPA 10X Safety Factor for Infants & Children	These points increase the uncertainty that the PoD based on the CCCEH cord blood data are not fully representative and have been used as support to retain the FQPA 10X Safety Factor.
Relatively moderate to large exposure differences needed to see significant effects, likely due to the modest sample sizes used.		
Exposure at one point in prenatal time with no additional information regarding postnatal exposures.		
Lack of clarity regarding a linear dose-response instead of a potential threshold effect.	7.2 Options for PoD Based on the CCCEH Biomonitoring Data	This remains an uncertainty since CCCEH investigators have not released the raw data publicly. However, given the modest sample size and that the voluntary cancellation occurred in 2000, the data may not support a different empirical model (i.e, non-linear model). This point adds support the selection of the 2% change in working memory as this value is within the measured data (albeit at the low end).
Use of a single or average sample for exposure. Although Whyatt *et al.* (2009) noted moderate but significant correlations between meconium and cord and maternal blood and average urine TCPy, the representativeness of a single point exposure is still unclear.	6.3 Residential Exposure to the CCCEH Cohort & 7.1 Uncertainties with Using Biomarker for the PoD	The agency notes similar uncertainties in Section 7.1. The agency has developed an analysis of the range of possible exposures to the CCCEH cohort. This analysis uses the best available information on pesticide use and chlorpyrifos applications from 1998-2004, including Whyatt et al (2003) which indicates that pesticide applications occurred

Uncertainty Identified by the FIFRA SAP (2012, p. 48)	Section Where Considered	Comment
Time-varying exposures or the ability to define cumulative exposures would be preferable.		approximately monthly. The agency's predicted blood concentrations closely match those reported by CCCEH.
Lack of specificity of a critical window of effect and the potential for misclassification of individual exposure measures.		
External generalizability of the cohorts given their unique racial/ethnic and socioeconomic characteristics. However, it should be noted that their exposures were within the range of those seen in NHANES.	8.1. Intra-species Extrapolation	The agency concurs there are remaining uncertainties about the degree to which the CCCEH is more or less sensitive to other population groups. Thus, this issue is part of the rationale for retaining the 10X intra-species factor.
Questions about biologic plausibility due to lack of clarity on mechanism of action, particularly at the low exposure levels seen in the cohorts and the limited and mixed results of animal studies showing neurodevelopmental effects.	Appendix 3.0 (3.1.1, 3.1.2, 3.2) Background & Summary of Experimental and Epidemiology Studies on Neurodevelopmental Effects & 6.3 Residential Exposure to the CCCEH Cohort	The agency agrees that the AOP/MOA for neurodevelopmental outcomes are not known. The agency has conducted a systematic review of the laboratory animal literature. There is qualitative similarity with respect to the three major neurodevelopmental domains (specifically, cognition, motor control, and social behavior) between the laboratory animal studies and the epidemiology findings. The CCCEH exposure characterization analysis provides plausibility to the low blood levels.

11.0 References (for main document & appendices)

Abduljalil, K., Furness, P., Johnson, T.N., Rostami-Hodjegan, A., Soltani, H., 2012. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin. Pharmacokinet. 51, 365–96.

Albers JW, Garabrant DH, Berent S, Richardson RJ. **2010**. Paraoxonase status and plasma butyrylcholinesterase activity in chlorpyrifos manufacturing workers. J Expo Sci Env Epid 20:79–100.

Aldridge, J. E., Levin, E. D., Seidler, F. J., & Slotkin, T. A. (2005). Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. *Environ Health Perspect, 113*(5), 527–531.

Alfonso-Loeches, S., & Guerri, C. (2011). Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. *Crit Rev Clin Lab Sci, 48*(1), 19–47.

American Congress of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine (ACOG). "Safe Prevention of the Primary Cesarean Delivery". March 2014. http://www.acog.org/Resources_And_Publications/Obstetric_Care_Consensus_Series/Safe_Prevention_of_the_Primary_Cesarean_Delivery

Andersen, Helle R., Debes, Fróði, Wohlfahrt-Veje, Christine, Murata, Katsuyuki, Grandjean, Philippe. (2015) Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. Neurotoxicology and Teratology 47:1–9.

Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. (2010) Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. Sci Total Environ. 408:790–795.

Barter ZE, Chowdry JE, Harlow JR, Snawder JE, Lipscomb JC, Rostami-Hodjegan A. (2008) Covariation of human microsomal protein per gram of liver with age: absence of influence of operator and sample storage may justify interlaboratory data pooling. Drug Metab Dispos 36:2405–2409.

Beamer, P; Canales, R; and Leckie, J. (2009) Developing probability distributions for transfer efficiencies for dermal exposure. Journal of Exposure Science and Environmental Epidemiology. 19: 274–283.

Berkowitz, G. S., Obel, J., Deych, E., Lapinski, R., Godbold, J., Liu, Z., Wolff, M. S. (2003). Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. Environ Health Perspect, 111(1), 79–84.

Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, Holzman IR, Wolff MS. (2004) In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference. Environmental Health Perspectives. 112:388–391.

Billauer-Haimovitch, H., Slotkin, T. A., Dotan, S., Langford, R., Pinkas, A., & Yanai, J. (2009). Reversal of chlorpyrifos neurobehavioral teratogenicity in mice by nicotine administration and neural stem cell transplantation. *Behav Brain Res, 205*(2), 499–504.

Black M[1], Bhattacharya S[1], Philip S[2], Norman JE[3], McLernon DJ[1] (2015) Planned Cesarean Delivery at Term and Adverse Outcomes in Childhood Health. *JAMA.* 2015; 314(21):2271–2279. doi:10.1001/jama.2015.16176.

Bradman, A., Whitaker, D., Quiros, L., Castorina, R., Claus Henn, B., Nishioka, M., et al. (2007). Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. J Expo Sci Environ Epidemiol, *17*(4), 331–349.

Brown, D. M., Lindsted SL, Rhomber LR, Belites RP, 1997. Physiological Parameter Values for Physiologically Based Pharmacokinetic Models,. Toxicology and Industrial Health, 13**,** 77.

Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides.Pediatrics. 2010 Jun; 125(6):e1270-7. doi: 10.1542/peds.2009–3058

Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., *et al.* (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect, 119(8), 1189–1195.

Budtz-Jørgensen, E; Keiding, N; Grandjean, P; *et al.* (1999) Methylmercury neurotoxicity independent of PCB exposure. [Letter]. Environ Health Perspect 107(5):A236–237.

Budtz-Jørgensen, E; Grandjean, P; Keiding, N; *et al.* (2000) Benchmark dose calculations of methylmercury-associated neurobehavioral deficits. Toxicol Lett 112-113:193–199.

Busby-Hjerpe, A. L., et al., 2010. Comparative pharmacokinetics of chlorpyrifos versus its major metabolites following oral administration in the rat. Toxicology. 268**,** 55–63.

Carr, R. L., & Chambers, J. E. (1996). Kinetic Analysis of the *in Vitro* Inhibition, Aging, and Reactivation of Brain Acetylcholinesterase from Rat and Channel Catfish by Paraoxon and Chlorpyrifos-oxon. Toxicology and Applied Pharmacology, Volume 139, Issue 2, August 1996, Pages 365–373.

Castoldi, A. F., Onishchenko, N., Johansson, C., Coccini, T., Roda, E., Vahter, M., et al. (2008). Neurodevelopmental toxicity of methylmercury: Laboratory animal data and their contribution to human risk assessment. *Regul Toxicol Pharmacol, 51*(2), 215–229.

Chambers, J.E. (2013). In vitro Sensitivity of Cholinesterase to Inhibition by Chlorpyrifos-oxon in Several Tissues of the Rat. College of Veterinary Medicine, Mississippi State University.

Chen, X.P., Chen, W.Z., Wang, F.S., Liu, J.X. (2012). Selective cognitive impairments are related to selective hippocampus and prefrontal cortex deficits after prenatal chlorpyrifos exposure. *Brain Res.* 1474:19–28.

Chen L, Zhao T, Pan C, Ross JH, Krieger RI. (2012) Preformed biomarkers including dialkylphosphates (DAPs) in produce may confound biomonitoring in pesticide exposure and risk assessment. J Agric Food Chem. Sep 12; 60(36):9342–51. doi: 10.1021/jf303116p. Epub 2012 Sep 4.

Cole, T. B., et al., 2005. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. Pharmacogenet.Genomics. 15, 589–598.

Cowles, A. L., Borgstedt, H.H. and Gillies, A. J. 1971. "Tissue weights and rates of blood flow in man for the prediction of anesthetic uptake and distribution," *Anesthesiology*, vol. 35, no. 5, pp. 523–526.

Das, K. P., & Barone, S., Jr. (1999). Neuronal differentiation in PC12 cells is inhibited by chlorpyrifos and its metabolites: is acetylcholinesterase inhibition the site of action? *Toxicol Appl Pharmacol, 160*(3), 217–230.

David M, Borde T, Brenne S, Henrich W, Breckenkamp J, Razum O (2015) Caesarean Section Frequency among Immigrants, Second- and Third-Generation Women, and Non-Immigrants: Prospective Study in Berlin/Germany. PLoS ONE 10(5): e0127489. doi:10.1371/journal.pone.0127489.

Davidson, P; Myers, G; Cox, C; *et al.* (1995) Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion: outcomes at 19 and 29 months. NeuroToxicology 16:677-688.

Davidson, PW; Myers, GJ; Cox, C; *et al.* (1998) Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles child development study. JAMA 280:701-707.

Dow AgroSciences, Dow Chemical Company Battelle Pacific Northwest National Laboratory. (2011). Source-to-Outcome Modeling Physiologically-Based Pharmacokinetic/Pharmacodynamic (PBPK/PD) Model linked to a Dietary Exposure Model: Chlorpyrifos as a Case Study. Prepared for the FIFRA Scientific Advisory Panel meeting on February 15–18, 2011 meeting: http://www.epa.gov/scipoly/sap/meetings/2010/index.html.

Dow AgroSciences (2014a). Memo from Paul Price dated October 1, 2014. Additional PBPK modeling to estimate 1% RBC AChE inhibition levels from simulated exposures to chlorpyrifos.

Dow AgroSciences (2014b). Memo from Paul Price dated October 29, 2014. Development of Chemical Specific Adjustment Factors for Chlorpyrifos and Chlorpyrifos Oxon Using Target Red Blood Cell Acetyl Cholinesterase Inhibition Levels of 10%, 5%, and 1%.

Drew, D., 11/18/14, D424486, Chlorpyrifos Acute and Steady State Dietary (Food Only) Exposure Analysis to Support Registration Review.

Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. (2007) Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral Assessment Scale in a Multiethnic Pregnancy Cohort. American Journal of Epidemiology. 165:1397–1404.

Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal Exposure to Organophosphates, Paraoxonase 1, and Cognitive Development in Childhood. (2011) Environmental Health Perspectives. 119:1182–1188.

Engel SM, Bradman A, Wolff MS, Rauh V, Harley KG, Yang JH, Hoepner LA, Barr DB, Yolton K, Vedar MG, Xu Y, Hornung RW, Wetmur JG, Chen J, Holland NT, Perera FP, Whyatt R, Lanphear BP, Eskenazi B. (2015) Prenatal Organophosphorus Pesticide Exposure and Child Neurodevelopment at 24 Months: An Analysis of Four Birth Cohorts. Environmental Health Perspectives. http://dx.doi.org/10.1289/ehp.1409474.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT. (2004) Association ofin Utero Organophosphate Pesticide Exposure and Fetal Growth and Length of Gestation in an Agricultural Population. Environmental Health Perspectives. 115:792–798.

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. (2007) Organophosphate Pesticide Exposure and Neurodevelopment in Young Mexican-American Children. Environmental Health Perspectives. 115:792–798.

Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., & Holland, N. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. Environ Health Perspect, 118(12), 1775–1781.

Eskenazi, Brenda; Chevrier, Jonathan; Rauch, Stephen A.; Kogut, K; Harley, KG; Johnson, C; Trujillo, C; Sjodin, A; Bradman, A. (2013). In Utero and Childhood Polybrominated Diphenyl Ether (PBDE) Exposures and Neurodevelopment in the CHAMACOS Study. Environmental Health Perspectives: 121(2), 257–262.

Farina, M., Rocha, J. B., & Aschner, M. (2011). Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. *Life Sci, 89*(15-16), 555–563.

FIFRA Scientific Advisory Panel. (2000a). "Dietary Exposure Evaluation Model (DEEM™) and DEEM™ Decompositing Software." Report from the FIFRA Scientific Advisory Panel Meeting of February 29–March 3, 2000. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.
Available: http://archive.epa.gov/scipoly/sap/meetings/web/html/022900_mtg.html

FIFRA Scientific Advisory Panel. (2000b). "Calendex™ Calendar-based Dietary and Non-Dietary Aggregate and Cumulative Exposure Software System." Report from the FIFRA Scientific Advisory Panel Meeting of September 26–29, 2000. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.
Available: http://archive.epa.gov/scipoly/sap/meetings/web/html/092600_mtg.html

FIFRA Scientific Advisory Panel. (2002). "Organophosphate Pesticides: Preliminary OP Cumulative Risk Assessment."

FIFRA Scientific Advisory Panel. (2008). "The Agency's Evaluation of the Toxicity Profile of Chlorpyrifos." Report from the FIFRA Scientific Advisory Panel Meeting of September 16-19, 2008. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.
Available: http://www.epa.gov/scipoly/sap/meetings/2008/091608_mtg.htm.

FIFRA Scientific Advisory Panel. (2010). February 2–4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

FIFRA Scientific Advisory Panel. (2011). "Chlorpyrifos Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK-PD) Modeling linked to Cumulative and Aggregate Risk Evaluation System (CARES)." Report from the FIFRA Scientific Advisory Panel Meeting of February 15-18, 2011. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.
Available: http://www.epa.gov/scipoly/sap/meetings/2011/index.html.

FIFRA Scientific Advisory Panel. (2012). "Scientific Issues Associated with Chlorpyrifos". FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available
at: http://www.epa.gov/scipoly/sap/meetings/2012/041012meeting.html.

Forsberg ND[1], Rodriguez-Proteau R, Ma L, Morré J, Christensen JM, Maier CS, Jenkins JJ, Anderson KA. (2011) Organophosphorus pesticide degradation product in vitro metabolic stability and time-course uptake and elimination in rats following oral and intravenous dosing. Xenobiotica. 2011 May; 41(5):422-9. doi: 10.3109/00498254.2010.550656. Epub 2011 Mar 29.

Fortenberry G.Z., Meeker J.D., Sanchez B.N., *et al.*, (2014). Urinary 3,5,6-tichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: Distribution, temporal variability, and relationship with child attention and hyperactivity. International Journal of Hygiene and Environmental Health 217 (2014) 405–412.

Furlong, Melissa A., Engel, Stephanie M., Boyd Barr, Dana, Wolff, Mary S. (2014) Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. Environment International 70:125–131.

Garabrant, D. H., Aylward, L. L., Berent, S., Chen, Q., Timchalk, C., Burns, C. J., *et al.* (2009). Cholinesterase inhibition in chlorpyrifos workers: Characterization of biomarkers of exposure and response in relation to urinary TCPy. J Expo Sci Environ Epidemiol, 19(7), 634–642.

Guodong D., Pei W., Ying T., Jun Z., *et al.*, (2012). Organophosphate Pesticide Exposure and Neurodevelopment in Young Shanghai Children. Environ Sci. Technol. 2012, 46, 2911–2917.

Grandjean, P; Weihe, P; White, R; et al. (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 20:1–12.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2007). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of Ecuador. Environ Health Perspect. 2007 Jan; 115(1): 128–33.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2007b). Neurobehavioral Development in Children with Potential Exposure to Pesticides. Epidemiology May 2007; 18: 312–320.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2008). Occupational Exposure to Pesticides During Pregnancy and Neurobehavioral Development of Infants and Toddlers. Epidemiology, Volume 19, number 6, November 2008.

Hill AB (1965). The Environment and Disease: Association or Causation? Proc R Soc Med. May 1965; 58(5): 295–300.

Hinderliter PM[1], Price PS, Bartels MJ, Timchalk C, Poet TS. 2011. Development of a source-to-outcome model for dietary exposures to insecticide residues: an example using chlorpyrifos. Regul Toxicol Pharmacol. 2011 Oct; 61(1):82–92.

Horton MK, Rundle A, Camann DE, Barr DB, Rauh VA, Whyatt RM. (2011). Impact of Prenatal Exposure to Piperonyl Butoxide and Permethrin on 36-Month Neurodevelopment. Pediatrics: 127(3), e699–706.

Hotchkiss JA, Krieger SM, Mahoney KM, *et al.* (2013). Nose-only inhalation of chlorpyrifos vapor: limited toxicokinetics and determination of time-dependent effects on plasma, red blood cell, brain and lung cholinesterase activity in female CD(SD): Crl rats. Report of The Dow Chemical Company.

Howard, A. S., Bucelli, R., Jett, D. A., Bruun, D., Yang, D., & Lein, P. J. (2005). Chlorpyrifos exerts opposing effects on axonal and dendritic growth in primary neuronal cultures. *Toxicol Appl Pharmacol, 207*(2), 112–124.

Hojring N, Svensmark O. (1976). J Neurochem. Carboxylesterases with defferent substrate specificity in human brain extracts. 1976 Aug; 27(2):525–8.

Icenogle, L. M., Christopher, N. C., Blackwelder, W. P., Caldwell, D. P., Qiao, D., Seidler, F. J., et al. (2004). Behavioral alterations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulation. *Neurotoxicol Teratol, 26*(1), 95–101.

Johansson, C., Castoldi, A. F., Onishchenko, N., Manzo, L., Vahter, M., & Ceccatelli, S. (2007). Neurobehavioural and molecular changes induced by methylmercury exposure during development. *Neurotox Res, 11*(3-4), 241-260

Janssen, I., *et al.*, 2000. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol. 89**,** 81–8.

Kisicki J.S., Seip, C.W., and Combs M.L. 1999. A Rising Dose Toxicology Study to Determine the No-Observable-Effect-Levels (NOEL) for Erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels. MDC Harris Laboratory, Lincoln Nebraska, Study No. 21438 (for the Harris Project) and DR K-0044793-284 (for Dow AgroSciences), April 19, 1999, MRID No. 44811002.

Kjellstrom, T; Kennedy, P; Wallis, S; *et al.* (1986) Physical and mental development of children with prenatal exposure to mercury from fish. Stage 1: Preliminary test at age 4. Natl Swed Environ Protec Bd, Rpt 3080 (Solna, Sweden).

Kjellstrom, T; Kennedy, P; Wallis, S; *et al.* (1989) Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. Natl Swed Environ Prot Bd, Rpt 3642 (Solna, Sweden).

Kousba, A. A., *et al.*, 2007. Age-related brain cholinesterase inhibition kinetics following *in vitro* incubation with chlorpyrifos-oxon and diazinon-oxon. Toxicological Sciences. 95**,** 147–155.

Krieger, R. I., C. E. Bernard, T. M. Dinoff, L. Fell, T. Osimitz, J. H. Ross, and T. Thongsinthusak. (2000). Biomonitoring and Whole Body Cotton Dosimetry to Estimate Potential Human Dermal Exposure to Semivolatile Chemicals. J. Exposure Analysis & Environ. Epidemiol. 10: 50–57.

Lafortuna, C. L., *et al.*, 2005. Gender variations of body composition, muscle strength and power output in morbid obesity. Int J Obes (Lond). 29**,** 833–41.

Lee, L. J. (2009). Neonatal fluoxetine exposure affects the neuronal structure in the somatosensory cortex and somatosensory-related behaviors in adolescent rats. Neurotox Res, 15(3), 212–223.

Levin, E. D., Addy, N., Nakajima, A., Christopher, N. C., Seidler, F. J., & Slotkin, T. A. (2001). Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. *Brain Res Dev Brain Res, 130*(1), 83–89.

Levin, E. D., Addy, N., Baruah, A., Elias, A., Christopher, N. C., Seidler, F. J., *et al.* (2002). Prenatal chlorpyrifos exposure in rats causes persistent behavioral alterations. *Neurotoxicol Teratol, 24*(6), 733–741.

Li B, Sedlacek M, Manoharan I, Boopathy R, Duysen EG, Masson P, Lockridge O. (2005). Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. Biochem Pharmacol. 2005 Nov 25; 70 (11):1673–84. Epub 2005 Oct 6.

Llop S., Julvez J., Marina L.S., Vizcaino E., *et al.*, (2013). Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. Environment International 59(2013) 175–182.

Lovasi, G. S., Quinn, J. W., Rauh, V. A., Perera, F. P., Andrews, H. F., Garfinkel, R., Rundle, A. (2011). Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. Am J Public Health, 101*(*1), 63–70.

Lowe, E. R., *et al.*, (2009). The Effect of Plasma Lipids on the Pharmacokinetics of Chlorpyrifos and the Impact on Interpretation of Blood Biomonitoring Data. Toxicological Sciences. 108**,** 258–272.

Lu, C., Holbrook, C. M., & Andres, L. M. (2010). The implications of using a physiologically based pharmacokinetic (PBPK) model for pesticide risk assessment. Environ Health Perspect, 118(1), 125–130.

Luecke, R. H., *et al.*, (2007). Postnatal growth considerations for PBPK modeling. Journal of Toxicology and Environmental Health-Part a-Current Issues. 70**,** 1027–1037.

Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environmental Health Perspectives* 2010; 118(12):1768–1774.

Marty, M. S., *et al.*, 2007. The effect of route, vehicle, and divided doses on the pharmacokinetics of chlorpyrifos and its metabolite trichloropyridinol in neonatal Sprague-Dawley rats. Toxicological Sciences. 100**,** 360–373.

Maxwell, D.M., Lenz, D.E., Groff, W.A., Kaminskis, A., Froehlich, H.L., 1987. The effects of blood flow and detoxification on *in vivo* cholinesterase inhibition by soman in rats. Toxicol. Appl. Pharmacol. 88, 66–76.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, Vickers C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. 2014 Jan;34(1):1–18.

MRID 44787301. Hoberman, A. (1999) Developmental Neurotoxicity Study of Chlorpyrifos Administered Orally via Gavage to Crl: CDBR VAF/Plus Presumed Pregnant Rats: Report Supplement 2: Lab Project Number: 301-001: K-044739–109. Unpublished study prepared by Argus Research Laboratories, Inc. 40 p.

MRID 49074901. Poet, T. (2013) Physiologically Based Pharmacokinetic/Pharmacodynamic (PBPK/PD) Modeling of Dermal Exposure to Chlorpyrifos: Validation and Application to Mixed Oral and Dermal Exposures. Project Number: NS000117. Unpublished study prepared by Battelle-Pacific Northwest Div. 71p.

MRID 49248201. Price, P.; Poet, T. (2013) Development of Chemical Specific Adjustment Factors for Chlorpyrifos and Chlorpyrifos Oxon. Project Number: NS000132. Unpublished study prepared by Dow Chemical Co. 55p.

MRID 49830302. Poet, T. (2016) Evaluation of the Dermal Delivery Compartment of the Chlorpyrifos PBPK Model. Project Number: NS000221, 160344, 10001558/001/10104/0027. Unpublished study prepared by Summit Toxicology. 54p.

Myers, GJ; Marsh, DO; Cox, C; *et al.* (1995a) A pilot neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet. Neurotoxicology 16(4):629–638.

Myers, GJ; Marsh, DO; Davidson, PW. (1995b) Main neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet: outcome at six months. Neurotoxicology 16(4):653–664.

Myers, GJ; Davidson, PW; Cox, C; et al. (1995c) Neurodevelopmetnal outcomes of Seychellois children sixty-six months after in utero exposure to methylmercury from a maternal fish diet: pilot study. Neurotoxicology 16(4):639–652.

Myers, GJ; Davidson, PW; Shamlaye, CF; et al. (1997) Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study. Neurotoxicology 18(3):819–830.

Neal, J.L, Lowe, N.K., Ahijevych, K.L, Patrick, T.E., Cabbage, L.A. and Corwin, E.J. (2010) 'Active labor' duration and dilation rates among low-risk, nulliparous women with spontaneous labor onset: a systematic review. J Midwifery Womens Health. 2010 Jul–Aug; 55(4): 308–318.

Needham, L. L. (2005). Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes. Environ Health Perspect, 113(4), 494–498.

Nolan, R. J., Rick, D. L., Feshour, M. L., & Saunders, J. H. (1982). Chlorpyrifos: Pharmacokinetics in human volunteers following single oral and dermal doses (MRID 00124144). The Dow Chemical Co. Biomedical Medical Research Laboratory. Toxicology Research Laboratory. Midland, MI.

Nolan, R. J., et al., 1984. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicology and Applied Pharmacology. 73, 8–15.

National Research Council (NRC, 2000) Toxicological Effects of Methylmercury, Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology Commission on Life Sciences, NATIONAL ACADEMY PRESS, Washington, DC. http://www.nap.edu/catalog/9899/toxicological-effects-of-methylmercury

National Research Council (NRC, 2009). Science and decisions: Advancing risk assessment. Washington, DC: The National Academies
Press. http://www.nap.edu/openbook.php?record_id=12209

National Research Council (NRC, 2011). "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde".

National Research Council (NRC, 2014). "Review of EPA's Integrated Risk Information System (IRIS) Process"

Ohishi T, Wang L, Akane H, Itahashi M, Nakamura D, Yafune A, Mitsumori K, Shibutani M. (2013). Reversible effect of maternal exposure to chlorpyrifos on the intermediate granule cell progenitors in the hippocampal dentate gyrus of rat offspring. Reprod. Toxicol. 35:125–136.

Oulhote, Y. and Bouchard, M.F. (2013) Urinary Metabolites of Organophosphate and Pyrethroid Pesticides and Behavioral Problems in Canadian Children. Environmental Health Perspectives. 121:1378–1384.

Perera FP[1], Rauh V, Tsai WY, Kinney P, Camann D, Barr D, Bernert T, Garfinkel R, Tu YH, Diaz D, Dietrich J, Whyatt RM. (2003) Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect. 2003 Feb; 111(2):201–5.

Petit, Claire, Chevrier, Cécile, Durand, Gaël, Monfort, Christine, Rouget, Florence, Garlantezec, Ronan, Cordier, Sylvaine. (2010) Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: a prospective cohort study in Brittany, France. Environmental Health 9:71-83.

Poet, T. S., –, 2003. In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. Toxicological Sciences: An Official Journal of the Society of Toxicology. 72**,** 193–200 %U http://www.ncbi.nlm.nih.gov/pubmed/12655035.

Poet, Torka S., Timchalk, C., Hotchkiss, Jon A., Bartels, M. J. (2014) Chlorpyrifos PBPK/PD model for multiple routes of exposure. Xenobioticam 2014; 44(10): 868–881

Pope, C. N., Karanth, S., Liu, J., & Yan, B. (2005). Comparative carboxylesterase activities in infant and adult liver and their in vitro sensitivity to chlorpyrifos oxon. Regul Toxicol Pharmacol, 42(1), 64–69.

Price PS[1], Conolly RB, Chaisson CF, Gross EA, Young JS, Mathis ET, Tedder. 2003 DR. Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit Rev Toxicol. 2003;33(5):469–503.

Price PS, Schnelle KD, Cleveland CB, Bartels MJ, Hinderliter PM, Timchalk C, Poet TS. 2011. Application of a source-to-outcome model for the assessment of health impacts from dietary exposures to insecticide residues. Regul Toxicol Pharmacol. 2011 Oct; 61(1):23–31.

Quiros-A.L., Alkom A.D., Boyce W.T., Lippert S., *et al.*, (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. NeuroToxicology 32 (2011) 646–655.

Racke J.D. Degradation of organophosphorous insecticidesin environmental matrices. In: Organophosphates Chemistry, Fate and Effects. Academic Press, Inc., San Diego, CA, 1992, pp 47–72.

Rauch, S.A., Braun, J.M., Boyd Barr, D., Calafat, A.M., Khoury, J. M., Montesano, A, Yolton, K, and Lanphear, B.P. (2012). Associations of Prenatal Exposure to Organophosphate Pesticide Metabolites with Gestational Age and Birth Weight. Environmental Health Perspectives. 120:1055–1060.

Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., Whyatt, R. W. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics, 118(6), e1845–1859.

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., & Whyatt, R. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect, 119(8), 1196–1201.

Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., Peterson, B. S. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc Natl Acad Sci U S A, 109(20), 7871–7876.

Rauh, Virginia A.; Garcia, Wanda E.; Whyatt, Robin M.; *et al.* (2015) Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. Neurotoxicology: 51, 80–86.

Ricceri, L., Markina, N., Valanzano, A., Fortuna, S., Cometa, M. F., Meneguz, A., *et al.* (2003). Developmental exposure to chlorpyrifos alters reactivity to environmental and social cues in adolescent mice. *Toxicol Appl Pharmacol, 191*(3), 189–201.

Roberts CL, Algert CS, Morris JM, *et al.* (2015) Increased planned delivery contributes to declining rates of pregnancy hypertension in Australia: a population-based record linkage study. BMJ Open 2015;5:e009313. doi:10.1136/bmjopen-2015-009313

Shelton, Janie F., Geraghty, Estella M., Tancredi, Daniel J., Delwiche, Lora D., Schmidt, Rebecca J., Ritz, Beate, Hansen, Robin L., and Hertz-Picciotto, Irva. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The CHARGE Study. Environmental Health Perspectives 122:1103–1109, 2014.

Sidell FR, Kaminskis A. (1975). Temporal intrapersonal physiological variability of cholinesterase activity in human plasma and erythrocytes. Clin Chem. 1975 Dec;21(13):1961–3.

Selim, S. (2004) Measurement of Transfer of Deltamethrin Residues from Vinyl and Carpet flooring Treated with a Fogger Formulation Following a Single Hand Press. Unpublished study prepared by Non-Dietary Exposure Task Force. (MRID 46297602).

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., Lazovich D., *et al.*, (2012). Lower acetylcholinesterase activity among children living with flower plantation workers. Environmental Research 114 (2012) 53–59.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., (2013). Aectylcholinesterase activity and neurodevelopment in boys and girls. PEDIATRICS Volume 132, number 6, December 2013.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., (2013a). Acetycholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian Children. Environmental Health Perspectives, volume 121, number 5, May 2013.

Smith JN, Hinderliter PM, Timchalk C, Bartels MJ, Poet TS. (2014) A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: development and validation. Regul Toxicol Pharmacol. 2014 Aug; 69(3):580-97. doi: 10.1016/j.yrtph.2013.10.005. Epub 2013 Nov 4.

Timchalk, C., *et al.*, 2002a. Monte Carlo analysis of the human chlorpyrifos-oxonase (PON1) polymorphism using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model. Toxicology Letters. 135**,** 51.

Timchalk, C., *et al.*, 2002b. A Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicological Sciences. 66**,** 34–53.

Timchalk, C., Poet, T. S., Hinman, M. N., Busby, A. L., & Kousba, A. A. (2005). Pharmacokinetic and pharmacodynamic interaction for a binary mixture of chlorpyrifos and diazinon in the rat. Toxicol Appl Pharmacol, 205(1), 31–42.

Timchalk, C., Poet, T. S., & Kousba, A. A. (2006). Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos. Toxicology, 220(1), 13–25

Timchalk, C., Poet, T. S., 2008. Development of a physiologically based pharmacokinetic and pharmacodynamic model to determine dosimetry and cholinesterase inhibition for a binary mixture of chlorpyrifos and diazinon in the rat. Neurotoxicology. 29**,** 428–443.

Trichilo, C., 1988, Chlorpyrifos Registration Standard (Second Round Review), 11/18/1988.

Toutain PL, Bousquet-Mélou A., (2004) Plasma terminal half-life, J. Vet. Pharmacol. Therap. 27, 427–439.

Turgeman, G., Pinkas, A., Slotkin, T. A., Tfilin, M., Langford, R., & Yanai, J. (2011). Reversal of chlorpyrifos neurobehavioral teratogenicity in mice by allographic transplantation of adult subventricular zone-derived neural stem cells. *J Neurosci Res, 89*(8), 1185–1193.

U.S. Environmental Protection Agency. (1999). Translation of Monitoring Data. HED Standard Operating Procedure 99.3. March 26, 1999.

U.S. Environmental Protection Agency. (2000). Human Health Risk Assessment: Chlorpyrifos. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C. Available at http://www.epa.gov/scipoly/sap/meetings/2008/september/hed_ra.pdf.

U.S. Environmental Protection Agency (2001) National Center for Environmental Assessment, Integrated Risk Information System (IRIS) Chemical Assessment Summary: Methylmercury (MeHg); CASRN 22967-92-6

U.S. Environmental Protection Agency. (2002). A review of the reference dose and reference concentration processes. December. Risk Assessment Forum, Washington, DC; EPA/630/P-02/002F. http://www.epa.gov/raf/publications/pdfs/rfd-final.pdf.

U.S. Environmental Protection Agency. (2004). An examination of EPA risk assessment principles and practices. Staff paper prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. Office of the Science Advisor, Washington, DC; EPA/100/B-04/001. http://www.epa.gov/osa/pdfs/ratf-final.pdf.

U.S. Environmental Protection Agency. (2005). Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Available at http://epa.gov/cancerguidelines/

U.S. Environmental Protection Agency. (2006a). Revised Organophosphorous Pesticide Cumulative Risk Assessment, July 31, 2006. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C. Available at http://www.epa.gov/pesticides/cumulative/rra-op/

U.S. Environmental Protection Agency. (2006b) Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (final report). U.S. Environmental Protection Agency, Washington, DC; EPA/600/R□05/043F.

U.S. Environmental Protection Agency. (2008). Science Issue Paper: Chlorpyrifos Hazard and Dose Response Characterization. August 21, 2008. Presented to the FIFRA SAP.

U.S. Environmental Protection Agency. (2010). Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. Environmental Protection Agency. (2011). Chlorpyrifos: Preliminary Human Health Risk Assessment for Registration Review. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0025.

U.S. Environmental Protection Agency. (2012a). Draft Issue Paper: Scientific Issues Concerning Health Effects of Chlorpyrifos. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2012-0040-0002

U.S. Environmental Protection Agency (2012b). J. Dawson, W. Britton, R. Bohaty, N. Mallampalli, and A. Grube. Chlorpyrifos: Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures. Office of Chemical Safety and Pollution Prevention. 7/13/12. D399483, D399485.

U.S. Environmental Protection Agency. (2012c). Benchmark Dose Technical Guidance. http://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf

U.S. Environmental Protection Agency. (2014a). Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review, December 29, 2014, D424485.

U.S. Environmental Protection Agency. (2014b). Chlorpyrifos Screening Level Usage Analysis (SLUA), May 1, 2014.

U.S. Environmental Protection Agency. (2014c). Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. http://www2.epa.gov/osa/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and

U.S. Environmental Protection Agency. (2014d). Chlorpyrifos: Quality Assurance Assessment of the Chlorpyrifos Physiologically Based Pharmacokinetic/Pharmacodynamic Model for Human Health Risk Assessment Applications. February 19, 2014, D417053.

U.S. Environmental Protection Agency. (2015) Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides. September 15, 2015, D331251.

Vaccaro (1991). Evaluation of Dislodgeable Residues and Absorbed Doses of Chlorpyrifos to Crawling Infants following of a Chlorpyrifos Based Emulsifiable Concentrate Indoor Broadcast Applications. MRID 42008401. Reviewed by EPA: D168824 8/18/1995 Valentin J (2002) In, Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values. Pergamon, Oxford.

Venerosi, A., Calamandrei, G., & Ricceri, L. (2006). A social recognition test for female mice reveals behavioral effects of developmental chlorpyrifos exposure. *Neurotoxicol Teratol, 28*(4), 466–471.

Venerosi, A., Ricceri, L., Rungi, A., Sanghez, V., & Calamandrei, G. (2010). Gestational exposure to the organophosphate chlorpyrifos alters social-emotional behaviour and impairs responsiveness to the serotonin transporter inhibitor fluvoxamine in mice. *Psychopharmacology (Berl), 208*(1), 99–107.

Verstraeten, S. V., Aimo, L., & Oteiza, P. I. (2008). Aluminium and lead: molecular mechanisms of brain toxicity. *Arch Toxicol, 82*(11), 789–802.

Wang, Pei, Tian, Ying, Wang, Xiao-Jin, Gao, Yu, Shi, Rong, Wang, Guo-Quan, Hu, Guo-Hua, Shen, Xiao-Ming. (2012) Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. 4 Environment International 2:100–104.

Watanabe, K. H., Andersen, M. E., Basu, N., Carvan, M. J., 3rd, Crofton, K. M., King, K. A., *et al.* (2011). Defining and modeling known adverse outcome pathways: Domoic acid and neuronal signaling as a case study. *Environ Toxicol Chem, 30*(1), 9–21.

Wechsler, D. (2003). Wechsler intelligence scale for children. 4th edition. San Antonio, TX: Psychological Corporation.

Whyatt RM[1], Camann DE, Kinney PL, Reyes A, Ramirez J, Dietrich J, Diaz D, Holmes D, Perera FP. (2002) Residential pesticide use during pregnancy among a cohort of urban minority women. Environ Health Perspect. 2002 May; 110(5):507–14.

Whyatt, R. M., Barr, D. B., Camann, D. E., Kinney, P. L., Barr, J. R., Andrews, H. F., Perera, F. P. (2003). Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect, 111(5), 749–756.

Whyatt, R. M., Rauh, V., Barr, D. B., Camann, D. E., Andrews, H. F., Garfinkel, R., Perera, F. P. (2004). Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect, 112(10), 1125–1132.

Whyatt, R. M., Garfinkel, R., Hoepner, L. A., Holmes, D., Borjas, M., Williams, M. K., Camann, D. E. (2007). Within- and between-home variability in indoor-air insecticide levels during pregnancy among an inner-city cohort from New York City. En*viron Health Perspect, 115(3*), 383–389.

Whyatt, R. M., Garfinkel, R., Hoepner, L. A., Andrews, H., Holmes, D., Williams, M. K., Barr, D. B. (2009). A biomarker validation study of prenatal chlorpyrifos exposure within an inner-city cohort during pregnancy. Environ Health Perspect, 117(4), 559–567.

Whyatt, R., & Rauh, V. (2011). [Chlorpyrifos Correspondence with Columbia Researchers: (1) Responses to Scientific Advisory Panel (SAP) comments (Whyatt and Rauh 2010), and (2) Responses to Dow AgroSciences inquiries (Whyatt 2010).].

Wolff, MS, Engel, S, Berkowitz, G, Teitelbaum, S, Siskind, J, Barr, DB, Wetmur, J. Prenatal Pesticide and PCB Exposures and Birth Outcomes. (2007). Pediatric Research 61(2):243–250.

Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, Barr DB, Furlong CE, Holland NT. (2005) Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. NeuroToxicology. 26:199–209.

Young, J. F., *et al.*, 2009. Human Organ/Tissue Growth Algorithms that Include Obese Individuals and Black/White Population Organ Weight Similarities from Autopsy Data. Journal of Toxicology and Environmental Health-Part a-Current Issues. 72**,** 527–540.

Zhang X, Driver JH, Li Y, Ross JH, Krieger RI. (2008) Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment.J Agric Food Chem. Nov 26; 56(22):10638–45. doi: 10.1021/jf8018084.

Zhang Y., Han S., Liang D., Shi X., Wang F., *et al.*, (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. Plos ONE 9(2):e88491. doi:10.1371/journal.pone.0088491.

Appendix 1.0: PoDs Based on 10% AChE Inhibition from the 2014 Human Health Risk Assessment

Summary information is provided below. Complete analyses and more detailed narratives are provided in the 2014 HHRA (USEPA, 2014a).

Like other OPs, chlorpyrifos binds to and phosphorylates the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. This can lead to accumulation of acetylcholine and, ultimately, at sufficiently high doses, to clinical signs of toxicity. In connection with EPA's FIFRA registration review of chlorpyrifos, and to address issues raised in an administrative petition seeking revocation of chlorpyrifos tolerances, EPA presented comprehensive reviews of the literature for AChE inhibition for the 2008 and 2012 SAP reviews along with the 2011 preliminary and 2014 revised risk assessment. AChE inhibition was the critical effect for the 2011 and 2014 risk assessments; this approach is consistent with the advice of the SAP from 2008 and 2012. The agency has conducted benchmark dose (BMD) analysis of numerous studies using empirical approaches previously accepted by the FIFRA SAP (USEPA, 2002) and consistent with the 2006 OP cumulative risk assessment (USEPA, 2006a) and other single chemical OP risk assessments. There are many chlorpyrifos studies evaluating AChE inhibition in red blood cell (RBC) or brain in multiple lifestages (gestational, fetal, post-natal, and non-pregnant adult), multiple species (rat, mouse, rabbit, dog, human), methods of oral administration (oral gavage with corn oil, dietary, gavage via milk) and routes of exposure (oral, dermal, inhalation via vapor and via aerosol). In addition, chlorpyrifos is unique in the availability of ChE data from peripheral tissues in some studies (*e.g.*, heart, lung, liver). There is also scientific literature comparing the *in vitro* ChE response to a variety of tissues (Chambers, 2013) which show similar sensitivity and intrinsic activity. Across the database, brain AChE tends to be less sensitive than RBC AChE or peripheral ChE. In oral studies, RBC AChE inhibition is generally similar in response to peripheral tissues.

In typical risk assessments, points of departures (PoDs) are derived directly from laboratory animal studies and inter- and intra-species extrapolation is accomplished by use of 10X factors. In the case of chlorpyrifos and its oxon, PBPK-PD modeling was used in 2014 HHRA to estimate PoDs for all age groups. The variation model was used to develop Data-Derived Extrapolation Factors (DDEF) for intra-species extrapolation for some groups (USEPA, 2014c) (See Section 4.8.4.1 in the 2014 HHRA). The agency typically uses a 10% response level for AChE inhibition in human health risk assessment. This response level is consistent with the 2006 OP cumulative risk assessment (USEPA, 2006a) and other single chemical OP risk assessments. As such, the model was used to estimate exposure levels resulting in 10% RBC AChE inhibition following single day (acute; 24 hours) and repeated exposures for a variety of exposure scenarios. The PBPK-PD model accounts for PK and PD characteristics to derive age, duration, and route specific PoDs. Separate PoDs were calculated for dietary (food, drinking water), residential, and occupational exposures by varying inputs on types of exposures and

populations exposed. Specifically, the following characteristics have been evaluated: duration [acute, 21-day (steady state)]; route (dermal, oral, inhalation); body weights which vary by lifestage; exposure duration (hours per day, days per week); and exposure frequency [events per day (eating, drinking)]. It should also be noted that PoDs were calculated for exposure patterns that would be considered protective of all others within each category (*e.g.*, children 1–2 years old are used to evaluate mosquito adulticide exposures for children because of their behavior they have high dermal exposures relative to their body weight and they also exhibit mouthing behaviors based on their stage of development).

Table 1.1. Table 4.8.4 from the 2014 Revised Human Health Risk Assessment: Chlorpyrifos PBPK Modeled Doses (PoDs) Corresponding to 10% RBC AChE Inhibition

Table 4.8.4. Chlorpyrifos PBPK Modeled Doses (PoDs) Corresponding to 10% RBC AChE Inhibition											
RA Type	Exposure Pathway (all chlorpyrifos unless noted)	Infants (<1 yr old)		Young Children (1–2 years old)		Children (Residential:6-11 years old; Dietary:6–12 years old)		Youths (Residential:11-16 years old; Dietary:13–19 years old)		Females (13–49 years old)	
		Acute	Steady State (21-day)	Acute	Steady State (21-day)	Acute	Steady State (21-day)	Acute	Steady State (21-day)	Acute	Steady State (21-day)
Dietary	Drinking Water (oxon conc, ppb)	1,183	217	3,004	548	7,700	1,358	4,988	878	5,285	932
	Food (µg/kg/day)	600	103	581	99	530	90	475	80	467	78
Residential (Golfers)	Dermal (µg/kg/day)						22,750		13,950		11,890
Residential (Mosquitocide Application)	Dermal (µg/kg/day)				134,250						23,600
	Oral (µg/kg/day)				101						
	Inhalation (concn. in air mg/m^3)				2.37						6.15
Occupational	Dermal (µg/kg/day)										3,630
	Inhalation (µg/kg/day)										138

*PoDs and exposure and risk estimates for females 13–49 yrs covers all youths >13 yrs

Predicted peak blood concentration at the external dose to achieve 10% RBC AChE inhibition for occupational dermal scenario, for example, is about 100,000 pg/g; and predicted blood concentration 10 hours after the peak is about 10,000 pg/g.

Table 1.2. Peak chlorpyrifos concentration in blood given the same steady state food exposure and worker dermal exposure scenarios used in the 2014 Revised Human Health Risk Assessment at the chlorpyrifos doses corresponding to 10% RBC AChE inhbition.

RA Type	**Exposure pathway**	**Infants (<1 year old)**	**Females (13 – 49 years old)**
Dietary	Food	6375 pg/g	7572 pg/g
Worker	Dermal		122,904 pg/g

Appendix 2.0: Summary of 2014 Dose Reconstruction Analysis

On April 10–12 2012, a FIFRA SAP meeting was held in review of, Scientific Issues Concerning Health Effects of Chlorpyrifos. Following the SAP meeting, the Panel provided the memorandum, Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held April 10–12, 2012 on "Chlorpyrifos Health Effects." The following was a recommendation of the SAP Panel for characterization of chlorpyrifos exposures:

"Environmental monitoring and biomonitoring data in the epidemiological studies contribute to the overall database on estimation of exposure, including (particularly) population variability and (to some degree) inter-individual variability in the study populations. They also provide insight into more generalizable observations on temporal trends in exposure of the general population — *e.g.*, following the impact of withdrawal of domestic (nonagricultural) uses of chlorpyrifos.

The biomonitoring and environmental monitoring data from the three children's cohort studies should be used, then, along with exposure information from other studies and sources, to characterize the levels of exposure to chlorpyrifos experienced in different 56 populations (production workers, agricultural workers, individuals exposed via residential use, general population, etc.), and in similar populations over time (*e.g.*, before and after cancellation of residential uses)."

Consistent with the recommendation of the SAP Panel, and in order to better understand the possible exposure patterns in the CCCEH study cohort and their influence on the health outcomes identified in the research, an analysis was conducted to supplement the exposure metrics reported by the CCCEH investigators. The goal was to predict potential high-end, or "screening level" exposures which could have occurred to pregnant women and young children who may have been exposed to chlorpyrifos through other pathways not addressed in the research then determine their potential for associated cholinesterase inhibition. Specifically, whether these screening level exposures could have resulted in 10% RBC AChE inhibition, the current regulatory endpoint for chlorpyrifos.

Chlorpyrifos, at the time, was one of the most widely used insecticides for indoor pest control so there are many possible ways which exposures could have occurred as a result of these types of uses (*e.g.*, use of a total release fogger, aerosol can sprays, or crack and crevice treatment by a pest control operator). This analysis was based on the agency's SOPs for Residential Exposure Assessment[51] which describe, on a scenario basis, specific algorithms and data used to predict the potential exposures which could have occurred to individuals in the cohort. Once complete, the resulting dose estimates were used to evaluate the potential level of cholinesterase inhibition which could be attributed to them using the PBPK-PD model. The scenarios which were considered include:

[51] http://www.epa.gov/pesticides/science/residential-exposure-sop.html

- Pregnant mothers who may have purchased a consumer aerosol can product and applied it in their homes;
- Exposures to pregnant mothers who may have had contact with residues in their homes after a previous treatment which leads to exposure; and
- Exposures to young children (aged 1 to 2 years old) who may have contact with residues in their homes after a previous treatment which leads to exposure.

Use Profile

In order to evaluate the chlorpyrifos exposure potential for the CCCEH study cohort, it was first necessary to define what chlorpyrifos products would have been in use during the time of study conduct which spanned from 1998 to 2004. In 1997, the registrant, Dow AgroSciences, voluntarily agreed to cancel chlorpyrifos registrations for indoor broadcast use and direct pet treatments, except pet collars. In December 2001, the majority of the remaining chlorpyrifos residential products - except for fully contained ant baits in child resistant packaging and limited public health uses - were subject to voluntary phase out/cancellation. This analysis reconstructed the potential exposures for those in the cohort between the years 1997–2001 based on the chlorpyrifos uses that remained after the voluntary cancellation in 1997 and the phase out in 2001. The exposure questionnaire-based reported use from the CCCEH publications was also used to inform this process. Per the publications, pest control measures were used by 85% of respondents, whether by housing superintendent, pest control operator (PCO), or by themselves. Of these, respondents reported that 39% were by PCO, 26% by spray can, and 5% foggers/bombs. The majority reported regular use of a pesticide product, at least once per month. Unfortunately, while these data help inform the types of products used, they do not allow for the determination of what pesticide active ingredient(s) were used. Multiple active ingredients were in regular use during this time period including, chlorpyrifos, diazinon, and propoxur, among others. For the purpose of the dose reconstruction exercise, it was assumed that only chlorpyrifos applications occurred. Considering all the available information, the potential for chlorpyrifos exposures from use of the following chlorpyrifos product types were assessed: broadcast ready to use (RTU) sprays applied by individual themselves and not professional applicators; surface sprays applied by individuals; crack and crevice sprays applied by individuals themselves or professional pest control applicators; and foggers/bombs applied by individuals. These chlorpyrifos residential products contained either 0.5% or 1% active ingredient. The latter was assumed to ensure an assessment reflective of the highest potential exposures, or those most likely to result in the greatest % RBC AChE inhibition.

Exposure Assessment Methods

With the products and application rates defined, the 2012 Standard Operating Procedures for Residential Pesticide Exposure Assessment[52] were used to assess the exposures that may have

[52] http://www.epa.gov/pesticides/science/USEPA-OPP-HED_Residential%20SOPs_Oct2012.pdf

occurred from these indoor residential chlorpyrifos uses. Based on the use patterns of the defined products, it is expected that exposures occurred either from the application (handling) of products by those in the cohort or after applications from being in contact with previously treated indoor environments.

A series of assumptions and exposure factors serve as the basis for completing residential handler and post-application exposure assessment:

- maximum application rates allowed by labels in its risk assessments are assumed;
- the use of personal protective equipment is not considered in residential risk assessments;
- residential assessments are based on the assumption that individuals are wearing shorts, short-sleeved shirts, socks, and shoes;
- adults are assumed to perform all pesticide applications;
- the mean female bodyweight reflective of all trimesters of pregnancy, 75 kg, was assumed for pregnant women to reflect the population of interest from the CCCEH cohort being evaluated which was derived from the EPA Exposure Factors Handbook 2011 Edition [53] (adult and adult female: Tables 8-3 through 8-5; body weight of pregnant women: Table 8-29).
- the most protective index lifestage which serves as the basis for evaluating young children's indoor exposures are children 1 to <2 years old as described in in the 2012 Residential SOPs;
- the body weight used to evaluate exposures to children 1 to <2 years old is 11 kilograms.

For residential handler exposure assessment, the following assumptions and exposure factors apply:

- a broadcast application of an aerosol can is considered and the entire contents of the can (16 oz.) are applied at once, half the contents of the spray can are applied for crack and crevice applications, and a quarter of the can contents for a space spray application.

For residential post-application exposure assessment, the following assumptions and exposure factors apply:

- inhalation exposures are expected to occur up to 2 hours following application by which time the aerosolized particulate is assumed to have settled and, as a result, after that time frame exposures are assumed to be negligible.

Use of the PBPK-PD Model

Once complete, the PBPK-PD model was used to estimate the potential level of cholinesterase inhibition (% RBC AChE) attributable to the daily dose estimates that result in the highest exposure potential. In order to model the potential health outcomes in a manner reflective of these exposures, a series of inputs were used that correspond to those used to quantify the daily dose estimates. The following exposure characteristics were used: number of days exposed;

[53] http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252

body weights which vary by lifestage; daily exposure duration (hours per day, days per week); and the fraction of the dermal surface area exposed.

The dose reconstruction exercise was modeled for a subsequent 14 day post-application duration. The daily dose estimated with the 2012 Standard Operating Procedures for Residential Pesticide Exposure Assessment for the day of application was inputted into the PBPK-PD model. Dermal exposure was modeled assuming that chlorpyrifos residues dissipated at a rate of 10% per day. A 14-day period was used as the basis of the PBPK-PD modeling to ensure it adequately addressed the time it takes for the ACHE enzyme inhibition to reach a steady state.

For the adult and children 1 to <2 years old age groups, the appropriate body weights were obtained for modeling purposes from the 2011 US EPA Exposure Factors Handbook.[54] The mean adult female bodyweight reflective of all trimesters of pregnancy, 75 kg, was assumed for pregnant women to reflect the population of interest from the CCCEH cohort being evaluated (Exposure Factors Handbook: adult and adult female: Tables 8-3 through 8-5; body weight of pregnant women: Table 8-29). For children 1 to <2 years old, the body weight was set to 11 kg (Exposure Factors Handbook, Table 8-3, mean body weight for the 1 to <2 year old age group). For the evaluation of adult handler exposures (dermal and inhalation) it was assumed that the application event continued for 1 hour daily and that it occurred each day of the 14 day exposure period. The 2012 Residential SOPs makes no recommendation regarding the daily exposure duration for handlers. It was assumed that 1 hour is a high end estimate of the time required to make an aerosol can application.

For the evaluation of adults and children 1 to <2 years old post-application exposures (*i.e.*, adults: dermal; children: dermal and incidental oral), the daily exposure duration was assumed to be 2 hours daily and that it occurs each day in the 14-day exposure period. This exposure duration is consistent with that recommended in the 2012 Residential SOPs for exposures on hard surfaces. For adults and children 1 to <2 years old inhalation exposures, it was assumed that the exposure duration was 2 hours on the day of application (Day 0) and was not repeated because the exposure source (*i.e.*, aerosols from an application event) occurred only once on the first day of the exposure period. This is consistent with the available monitoring data and consistent with the 2012 Residential SOPs, the Day 0 air monitoring measures were followed by a marked drop to negligible levels by the following day.

For all dose reconstruction dermal exposures to chlorpyrifos, the fraction of skin in contact with chlorpyrifos was 50% which reflects uncovered skin areas for children wearing shorts and a tee shirt. No shower (i.e., washing off the chlorpyrifos) was assumed to occur for the 14-day exposure duration modeled.

[54] http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252

Residential Handler Exposure Scenarios

The residential handling of chlorpyrifos residential products are expected to have occurred for some percentage of the CCCEH cohort population, these are anticipated to have resulted in dermal and inhalation exposures. Dermal and inhalation residential handler exposures are predicted by use of a factor known as the unit exposure. Unit exposure is the ratio, for a given formulation and application equipment, of an individual's exposure the amount of active ingredient handled (AaiH), expressed as mass active ingredient exposure per mass active ingredient handled (e.g., mg/lb a.i.). For the dose reconstruction assessment, unit exposure data were used as recommended in the 2012 Residential SOPs for ready-to-use (RTU) aerosol cans (dermal, 370 mg/ lb a.i.; inhalation, 3.0 mg/lb a.i.). Residential handler exposures from application of fogger formulations were not assessed which is consistent with the 2012 Residential SOPs. Fogger product labels typically direct the user to activate the device and evacuate the treated area and not to re-enter for several hours. Therefore, it is assumed that handler exposure from fogger application is not expected.

The exposure assessment for chlorpyrifos CCCEH cohort handler exposures is based on the following exposure scenarios:

- Adult dermal exposure from broadcast application of a RTU spray can;
- Adult inhalation exposure from broadcast application of a RTU spray can;
- Adult dermal exposure from crack and crevice application of a RTU spray can;
- Adult inhalation exposure from crack and crevice application of a RTU spray can;
- Adult dermal exposure from space spray application of a RTU spray can;
- Adult inhalation exposure from space spray application of a RTU spray can.

Handler Exposure Algorithm

Daily dermal and inhalation exposure (mg/day) for residential pesticide handlers, for a given formulation-application method combination, is estimated by multiplying the formulation-application method-specific unit exposure by an estimate of the amount of active ingredient handled in a day, using the equation below:

$$E = UE * AR * A$$

where:
E = exposure (mg/day);
UE = unit exposure (dermal, 370 mg/ lb ai; inhalation, 3.0 mb/lb ai);
AR = application rate (e.g., lb ai/can); and
A = area treated or amount handled (broadcast, 1 can/day; crack and crevice, 0.5 can/day; space spray, 0.25 can/day).

Residential Handler Exposure/Cholinesterase Inhibition Summary

Worst-case results of this analysis are presented below representing pregnant women who potentially made a broadcast application of an aerosol spray can (Table A.2.1). Peak % RBC AChE inhibition estimated for pregnant women from combined dermal and inhalation exposures occurring from product application is 0.0012%.

Table A.2.1. Residential Handler (Pregnant Women in the CCCEH Cohort) Estimated Exposures and Predicted % ChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Amount Handled	Dermal Exposure (mg/ day)	Dermal: Peak % ChE Inhibition	Inhalation Exposure (mg/day)	Inhalation: Peak % ChE Inhibition	Combined Peak % ChE Inhibition
Broadcast	Ready to Use: 1 % Aerosol Spray Can	1 can	3.7	0.0007%	0.030	0.0008%	0.0012%

a. Dermal Exposure = Dermal Unit Exposure (370 mg/lb a.i.) × Application Rate (0.010 lb a.i./can) × Area Treated or Amount Handled (cans/day)

b. Inhalation Exposure = Inhalation Unit Exposure (3.0 mg/lb a.i.) × Application Rate (0.010 lb a.i./can) × Area Treated or Amount Handled (cans/day)

Residential Post-application Exposure Scenarios

The use of indoor residential chlorpyrifos products are anticipated to have resulted in the potential for post-application exposures from public housing residents in the CCCEH study cohort being in a previously treated environment. Based on the product use profile, residents may have been exposed via inhalation to the aerosols resulting from application or by means of contact following settling of product contents following settling on indoor surfaces or flooring.

The exposure assessment for chlorpyrifos CCCEH cohort post-application exposures is based on the following exposure scenarios:

- Adult dermal contact with residues deposited on flooring (carpeted and hard) from broadcast application of RTU spray can;
- Adult inhalation of aerosolized particulates following broadcast application of RTU spray can;
- Adult dermal contact with residues deposited on flooring (carpeted and hard) from space spray application of RTU spray can;
- Adult inhalation of aerosolized particulates following space spray application of RTU spray can;
- Adult dermal contact with residues deposited on flooring (carpeted and hard) from crack and crevice RTU spray can or PCO application;
- Adult inhalation of aerosolized particulates following crack and crevice application of RTU spray can;

- Children 1 to <2 years old dermal contact with residues deposited on flooring (carpeted and hard) from broadcast application of RTU spray can;
- Children 1 to <2 years old incidental oral (hand-to-mouth) ingestion of residues deposited on flooring (carpeted and hard) from broadcast application of RTU spray can that have transferred to children's hands;
- Children 1 to <2 years old incidental oral (object-to-mouth) ingestion of residues deposited on toys in contact with flooring (carpeted and hard) treated by broadcast application of RTU spray can;
- Children 1 to <2 years old inhalation of aerosolized particulate following broadcast application of RTU spray can;
- Children 1 to <2 years old dermal contact with residues deposited on flooring (carpeted and hard) from space spray application of RTU spray can;
- Children 1 to <2 years old incidental oral (hand-to-mouth) ingestion of residues deposited on flooring (carpeted and hard) from space spray application of RTU spray can that have transferred to children's hands;
- Children 1 to <2 years old incidental oral (object-to-mouth) ingestion of residues deposited on toys in contact with flooring (carpeted and hard) treated by space spray application of RTU spray can;
- Children 1 to <2 years old inhalation of aerosolized particulate following space spray application of RTU spray can;
- Children 1 to <2 years old dermal contact with residues deposited on flooring (carpeted and hard) from crack and crevice RTU spray can or PCO applications;
- Children 1 to <2 years old incidental oral (hand-to-mouth) ingestion of residues deposited on flooring (carpeted and hard) from crack and crevice RTU spray can or PCO applications that have transferred to children's hands;
- Children 1 to <2 years old incidental oral (object-to-mouth) ingestion of residues deposited on toys in contact with flooring (carpeted and hard) treated by crack and crevice RTU spray can or PCO applications.

Since all exposure routes (dermal, inhalation, and incidental oral) share a common toxicological endpoint, RBC AChE inhibition, risk estimates should been combined. The incidental oral scenarios (i.e., hand-to-mouth and object-to-mouth) should be considered inter-related and it is likely that they occur interspersed amongst each other across time. Combining these scenarios with the dermal exposure scenario would be unrealistic because of the conservative nature of each individual assessment. Therefore, the post-application exposure scenarios that were combined for children 1 to <2 years old are the dermal and hand-to-mouth scenarios. This combination should be considered a protective estimate of children's exposure to pesticides used indoors.

The lifestages selected for each post-application scenario are based on an analysis provided as an Appendix in the 2012 Residential SOPs. While not the only lifestage potentially exposed for these post-application scenarios, the lifestage that is included in the quantitative assessment is health protective for the exposures and risk estimates for any other potentially exposed lifestage.

Post-application Exposure Data

The 2012 Residential SOPs relied on exposure data from agency submitted studies in order to establish many of the inputs necessary for estimation of post-application indoor exposures. Where chemical-specific exposure data are available, these data were relied upon as a more accurate measure of exposures from chlorpyrifos and are described below. In the absence of these data, exposure inputs are based on the use of available exposure data as recommended in the 2012 Residential SOPs.

Exposure Data Used for Dermal and Incidental Oral Exposure Assessment: No chemical-specific deposition data were available for the indoor chlorpyrifos products assessed. Therefore, as recommended by the 2012 Residential SOPs, default deposited residue values were used based on the type of application to be made whether it be a broadcast, crack and crevice, or space spay application. For broadcast applications only, residue values were influenced by the percent spray applied (*i.e.*, the higher the percent spray, the higher the residue values). The higher, 1% chlorpyrifos spray formulation was assumed for post-application assessment of broadcast exposures. The default values used are based on an analysis of available residue deposition data from agency submitted studies and literature studies. A summary of the recommended values are presented in the 2012 Residential SOPs.

The transfer coefficent (TC) input used for calculation of post-application exposures is a measure of surface-to-skin residue transfer derived from concurrent measures of exposure and surface residue. Per the 2012 Residential SOPs, no studies were available that measure both exposure and surface residue with subjects performed typical indoor activities. Therefore, the transfer coefficients used for indoor scenarios are derived from information provided in three different studies: 1) two studies which measured exposure and surface residues while subjects performed a Jazzercise routine (Krieger, 2000; Selim, 2004) and 2) a study which measured biomonitoring doses which adults performed scripted activities for 4 hours on carpets (Vaccaro, 1991). Of these studies, the Krieger study was conducted using chlorpyrifos.

As for the fraction of residue available for transfer input, a complete dataset was compiled for chlorpyrifos based on available chemical-specific data (Beamer *et al.*, 2009). Therefore, the chlorpyrifos-specific residue fractions anticipated to transfer for both carpets and hard surfaces were used for estimation of post-application dermal and incidental oral exposures.

Exposure Data Used for Inhalation Exposure Assessment: In order to best represent the potential for inhalation exposures following use of the indoor chlorpyrifos products assessed, a review of available literature studies and registrant-submitted exposure data was conducted. The following exposure data sources were identified and used:

Indoor broadcast applications:

- Fenske, R., *et al.* (1990). Potential Exposure and Health Risks of Infants following Indoor Residential Pesticide Applications. American Journal of Public Health. 80 (6): 689–693.
- Lu, C. and Fenske, R (1998). Air and Surface Chlorpyrifos Residues following Residential Broadcast and Aerosol Pesticide Applications. Environmental Science and Technology. 32 (10): 1386–1390.
- EPA MRID: 42887201. Contardi, J. (1993). An Evaluation of the Appropriate Drying Time via Air Monitoring, Dislodgable Residue Determination, and Carpet Weight Loss, After Applying Dursban LO Insecticide to a Carpeted Surface: Unpublished study prepared by Dow Chemical Co., Health and Environmental Sciences. 29 pp.

Indoor crack and crevice applications:

- Byrne, S.L., Shurdut, B.A. and Saunders, D.G. (1998). Potential Chlorpyrifos Exposure to Residents following Standard Crack and Crevice Treatment. 106 (11): 725–731.
- Hore, P. *et al.* (2005). Children's Residential Exposures to Chlorpyrifos: Application of CPPAES Field Measurements of Chlorpyrifos and TCPy within MENTOR/SHEDS — Pesticides Model. Science of the Total Environment. 336 (2–3): 525–537.
- Stout II, D.M. and Mason, M.A. (2003). The Distribution of Chlorpyrifos following a Crack and Crevice Type Application in the US EPA Indoor Air Quality Research House. Atmospheric Environment. 37 (39–40): 5539–5549.
- EPA MRID 44458201: Byrne, S.; Saunders, D.; Cook, W. *et al.* (1998) Residential Exposure to Chlorpyrifos from Reentry to Structures Treated with Crack and Crevice and Spot Applications of Dursban Pro. Unpublished study prepared by Dow AgroSciences. 133 pp.

Based on a review of the available data and consistent with the 2012 Residential SOPs, the day of application (Day 0) air monitoring measures were significant, followed by a marked drop to negligible levels by the following day. Therefore, HED made use only of day of application air monitoring measures for purpose of post-application exposure assessment. All studies referenced were based on 0.5% chlorpyrifos formulated products. In order to address the potential for use of the 1% chlorpyrifos product formulations available at the time, all air monitoring measures were doubled. The broadcast exposure data are intended for use in assessing the potential inhalation exposures/doses anticipated to occur from both broadcast and space spray applications; the crack and crevice exposure data were used only for that application type. The resulting mean day of application air monitoring measures are presented in Table A.2.2 below.

Table A.2.2. Airborne Concentrations of Chlorpyrifos from Open Literature and Registrant-submitted Studies Resulting from Indoor Broadcast and Crack and Crevice Applications	
Study	Chlorpyrifos Concentration (mg/m^3)
Broadcast	
Fenske, R., *et al.* (1990)	0.16
Lu, C. and Fenske, R (1998)	0.088
EPA MRID: 42887201	0.031
Average (All Studies)	0.092
Crack and Crevice	
Byrne, S.L., Shurdut, B.A. and Saunders, D.G. (1998)	0.0010
Hore, P. *et al.* (2005).	0.00057
Stout II, D.M. and Mason, M.A. (2003)	0.00086
EPA MRID 44458201	0.0011
Average (All Studies)	0.00089

As described above (Use of the PBPK-PD Model), dermal post-application exposures were modeled assuming that chlorpyrifos residues dissipated at a rate of 10% per day. This value is based on an evaluation of all available chlorpyrifos-specific floor residue data. Although seven exposure studies were identified above for sources of indoor air monitoring data, three were excluded from the dermal post-application exposure analysis because they were determined to be inadequate for the collection of daily residue dissipation from flooring. The four studies in the analysis included two registrant-submitted exposure studies and two exposure studies from the open literature. Of these four studies, only two measured floor residues beyond two subsequent days (*i.e.*, the day of product application and the day immediately following). This is relevant since in all four exposure studies, floor residues drop markedly (43–92% dissipation) on the day following product application, whereas the two exposure studies where measurements of floor residue were taken for more than two days, residues were observed to drop markedly initially, and then plateau. Therefore, to include in the dissipation analysis the exposures studies with only two days of residue measurements would overestimate residue dissipation and have the result of underestimating daily post-application exposures. Further, of the two exposure studies which measured floor residues for more than two days, only one measured residues for subsequent days which is necessary for accurate quantification of daily dissipation. Ultimately, the assumption of 10% daily dissipation is based the exposure study, Lu and Fenske (1998). While the reliance on a single exposure study dataset is limiting, the selection of 10% daily dissipation for PBPK modeling is expected to result in a health protective estimation of daily post-application exposures; that is, the lower the daily dissipation, the greater the amount of floor residues present and, likewise, greater predicted daily exposures. Table A.2.3 below presents daily floor residue dissipation resulting from all exposure studies, the number of days measured, and for those studies excluded, the reasoning for their exclusion.

Table A.2.3. Percent Daily Floor Residue Dissipation from Open Literature and Registrant-submitted Studies Resulting from Indoor Applications of Chlorpyrifos		
Study	Daily Floor Residue Dissipation (Mean %)	Days Measured
Multi-Day Floor Residue Measures		
Lu, C. and Fenske, R (1998) — Broadcast Application	10	1 – 7 (daily)
Hore, P. *et al.* (2005) — Crack & Crevice Application	33	1, 2, 3, 5, 7, 9, 11
Two-Day Floor Residue Measures		
EPA MRID: 42887201	75	1, 2
Fenske, R., *et al.* (1990) — Broadcast Application	80	1, 2
Excluded Exposure Studies		
Byrne, S.L., Shurdut, B.A. and Saunders, D.G. (1998)	Floor residues collected by carpet drags were reported to be negligible.	
EPA MRID 44458201	Carpet wipes all resulted in non-detectable residues.	
Stout II, D.M. and Mason, M.A. (2003)	Deposition coupons were used on floors, but not via direct application of the coupons.	

Post-Application Algorithms for All Scenarios

Post-Application Dermal Exposure Algorithm (hard surfaces and carpets)

The algorithm to calculate exposure is as follows:

where:

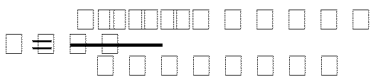
E = exposure (mg/day);
TR = indoor surface transferable residue (µg/cm2);
TC = transfer coefficient (cm2/hr);
ET = exposure time (hr/day); and
CF1 = conversion factor (0.001 mg/µg).

If chemical-specific TR data are available, this is preferred and should be used to calculate exposure. However, if chemical-specific TR data are not available, then TR can be calculated using the following formula:

where:

TR = indoor surface transferable residue (µg/cm2);
DepR = deposited residue (µg/cm2), based on (in order of preference):
(1) Chemical-specific residue deposition data (µg/cm2),
(2) Application rate (lb ai/area), or
(3) Default residue based on type of application (µg/cm2); and
Fai = fraction of a.i. available for transfer from carpet or hard surface (unitless).

Absorbed dermal dose, normalized to body weight, are calculated as:



where:
D = dose (mg/kg-day);
E = exposure (mg/day);
AF = absorption factor; and
BW = body weight (kg).

Table A.2.4-1. Indoor Environments (Hard Surfaces and Carpets) – Inputs for Residential Post-application Dermal Exposure				
Algorithm Notation	Exposure Factor (units)			Point Estimate(s)
TR	Transferable residue ($\mu g/cm^2$)			Estimated: DepR * Fai
DepR	Deposited residue ($\mu g/cm^2$)			Estimated based on default residue related to type of application
Fai	Fraction of DepR as TR following application	Carpets		0.020[a]
		Hard surfaces		0.13[a]
TC	Transfer Coefficient (cm^2/hr)	Adult		6,800
		Children 1 < 2 years old		1,800
ET	Exposure Time (hrs/day)	Adults	Carpets	8
			Hard Surfaces	2
		Children 1 < 2 years old	Carpets	4
			Hard Surfaces	2
BW	Body weight (kg)	Adult		75
		Children 1 < 2 years old		11

a. Chlorpyrifos-specific as identified (Table 7-8) in the 2012 Residential SOPs.

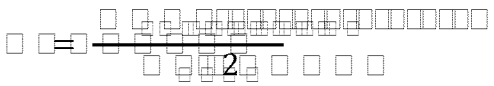
Post-application Hand-to-Mouth Exposure Algorithm

Exposure from hand-to-mouth activity is calculated as follows (based on algorithm utilized in SHEDS-Multimedia):

where:

E	= exposure (mg/day);
HR	= hand residue loading (mg/cm^2);
FM	= fraction hand surface area mouthed / event (fraction/event);
ET	= exposure time (hr/day);
SAH	= surface area of one hand (cm^2);
N_Replen	= number of replenishment intervals per hour (intervals/hour);
SE	= saliva extraction factor (*i.e.*, mouthing removal efficiency); and
Freq_HtM	= number of hand-to-mouth contacts events per hour (events/hour).

and



where:

HR	= hand residue loading (mg/cm^2);
Faihands	= fraction a.i. on hands compared to total surface residue from jazzercise study (unitless);
DE	= dermal exposure (mg); and
SAH	= typical surface area of one hand (cm^2).

Table A.2.4-2. Indoor Environments – Inputs for Residential Post-application Hand-to-Mouth Exposure		
Algorithm Notation	Exposure Factor (units)	Point Estimate(s)
Faihands	Fraction of a.i. on hands from jazzercise study (unitless)	0.15
DE	Dermal exposure calculated (mg)	Calculated
HR	Residue available on the hands (mg/cm^2)	Calculated

Table A.2.4-2. Indoor Environments – Inputs for Residential Post-application Hand-to-Mouth Exposure				
Algorithm Notation	Exposure Factor (units)			Point Estimate(s)
SAH	Surface area of one hand (cm^2)	Children 1 < 2 years old		150
AR	Application rate (mass active ingredient per unit area)			1% formulation
FM	Fraction of hand mouthed per event (fraction/event)			0.13
N_Replen	Replenishment intervals per hour (intervals/hr)			4
ET	Exposure time (hours per day)	Children 1 < 2 years old	Carpets	4
			Hard Surfaces	2
SE	Saliva extraction factor (fraction)			0.48
Freq_HtM	Hand-to-mouth events per hour (events/hr)	Children 1 < 2 years old		20
BW	Body Weight (kg)	Children 1 < 2 years old		11

Post-application Object-to-Mouth Exposure Algorithm

Exposure from object-to-mouth activity is calculated as follows (based on algorithm utilized in SHEDS-Multimedia):

$$E = OR \times CF1 \times SAMO \times (ET \times N\_Replen) \times [1 - (1 - SE)^{Freq\_OtM}]$$

where:

E	=	exposure (mg/day);
OR	=	chemical residue loading on an object (μg/cm^2);
CF1	=	weight unit conversion factor (0.001 mg/μg);
SAMO	=	area of the object surface that is mouthed (cm^2/event);
ET	=	exposure time (hr/day);
N_Replen	=	number of replenishment intervals per hour (intervals/hour);
SE	=	saliva extraction factor (*i.e.*, mouthing removal efficiency); and
Freq_OtM	=	number of object-to-mouth contact events per hour (events/hour).

and

$$OR = DepR \times FO$$

where:

OR = chemical residue loading on the object (µg/cm^2);
DepR = deposited residue (µg/cm^2); and
FO = fraction of residue transferred to an object (unitless).

Table A.2.5. Indoor Environments – Inputs for Residential Post-application Object-to-Mouth Exposure				
Algorithm Notation	Exposure Factor (units)			Point Estimate(s)
AR	Application rate (mass active ingredient per unit area)			[input]
FO	Fraction of residue transferred to an object	Carpets		0.020a
		Hard surfaces		0.13[a]
SAMO	Surface area of object mouthed (cm2/event)			10
N_Replen	Replenishment intervals per hour (intervals/hour)			4
SEO	Saliva extraction factor			0.48
ET	Exposure Time (hours per day)	Children 1 < 2 years old	Carpets	4
			Hard Surfaces	2
Freq_OtM	Object-to-mouth events per hour (events/hour)	Children 1 < 2 years old		14
BW	Body Weight (kg)	Children 1 < 2 years old		11

a. Chlorpyrifos-specific as identified (Table 7-8) in the 2012 Residential SOPs.

The results of this analysis are presented below for pregnant women who were potentially exposed from contact to residues which occur in previously treated areas such as their homes (Table A.2.6). Results for young children (aged 1 to < 2 years old) who are also exposed to residues which occur in previously treated areas such as their homes are presented in Table A.2.7. Peak cholinesterase inhibition for pregnant women resulting from combined dermal and

inhalation exposures occurring from contact with previously treated indoor areas is 0.45%. For children 1 to <2 years old, peak cholinesterase inhibition associated with combined dermal, incidental oral, and inhalation exposures from being in previously treated areas is 2.7%.

Table A.2.6. Residential Post-application To Pregnant Women in the CCCEH Cohort Estimated Dermal and Inhalation Exposures and Predicted % ChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Dermal Exposure (mg/day)	Dermal: Peak % ChE Inhibition	Airborne Concentration of Chlorpyrifos (mg/m3)	Inhalation: Peak % ChE Inhibition	Combined: Peak % ChE Inhibition
Broadcast (Hard Surfaces)	1% PCO Application or RTU 1 16 oz can	53	0.45%	0.092	0.0049%	0.45%

* See algorithms and inputs above used to estimate post-application dermal exposures.

Table A.2.7. Residential Post-application (Children 1 to < 2 Years Old in the CCCEH Cohort) Estimated Dermal and Inhalation Exposures and Predicted % RBC AChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Dermal Exposure (mg/day)	Dermal: Peak % ChE Inhibition	HTM Dose (mg/kg/day)	HTM Peak % ChE Inhibition (mg/day)	Airborne Concn. of Chlorpyrifos (mg/m^3)	Inhalation: Peak % ChE Inhibition	Combined: Peak % ChE Inhibition
Broadcast (Hard Surfaces)	1% PCO Application or RTU 1, 16 oz can	14	0.14%	0.10	2.2%	0.092	0.014%	2.7%

* See algorithms and inputs above used to estimate post-application dermal, (HTM) hand-to-mouth, and (OTM) object-to-mouth exposures/doses.

Appendix 3.0: Background & Summary of Experimental and Epidemiology Studies on Neurodevelopmental Effects

A.3.1 Neurodevelopmental Effects

The agency has taken a stepwise, objective and transparent approach in evaluating, interpreting, and characterizing the strengths and uncertainties associated with all of the available lines of scientific information related to the potential for adverse neurodevelopmental effects in infants and children. The stepwise evaluation began with the September 2008 FIFRA Scientific Advisory Panel (SAP) meeting involving a preliminary review of the literature for chlorpyrifos, with a particular focus on women and children (USEPA, 2008), followed by the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" for integration of epidemiology with other types of experimental data (USEPA, 2010; FIFRA SAP 2010). After the draft framework (2010) was published, the agency released "Chlorpyrifos: Preliminary Human Health Risk Assessment for Registration Review," focusing on the AChE inhibiting potential of chlorpyrifos (USEPA, 2011). This focus was consistent with the recommendation from the 2008 SAP that AChE data provide the most appropriate endpoint and dose-response data for deriving PoDs for purposes of risk assessment. In 2012, the agency convened another meeting of the FIFRA SAP focused on chlorpyrifos which incorporated the newest experimental data related to AChE inhibition and both cholinergic and non-cholinergic adverse outcomes, including neurodevelopmental studies on behavior and cognition effects (FIFRA SAP 2012). Similarly, the agency also performed a more in-depth analysis of the biomonitoring data and of epidemiological studies from three major children's health epidemiology cohort studies in the U.S., as well as plausible hypotheses on MOAs/AOPs leading to neurodevelopmental outcomes (USEPA 2012a). Following the 2012 SAP meeting, the agency solicited additional input from federal experts in the areas of Magnetic Resonance Imaging (MRI) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological studies.[55] In December, 2014, the agency released "Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review" which included the use of a PBPK-pharmacodynamic (PBPK-PD) model to derive human PoDs, which obviated the need for the animal to human extrapolation factor, and refined intra-species factors for some lifestages (USEPA 2014a). The chlorpyrifos 2014 revised HHRA also included retention of the 10X FQPA Safety Factor due to uncertainty regarding the degree of protection the endpoint of AChE inhibition provides for potential neurodevelopmental effects (USEPA, 2014a).

[55] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170

A.3.1.1 Mechanistic Studies on Adverse Outcome Pathway

A review of the scientific literature on potential MOA/AOP leading to effects on the developing brain was conducted for the 2012 FIFRA SAP meeting (USEPA, 2012a) and updated for the 2014 chlorpyrifos revised HHRA (USEPA 2014a). There is strong evidence that developmental neurotoxicity of chlorpyrifos may not be due to AChE inhibition *per se*, but to other actions on critical aspects of neuronal development. Following several decades of research, there is now a number of biologically plausible molecular events proposed for chlorpyrifos (and other OPs) effects on the developing nervous system, with ongoing research pursuing many of these potential pathways. Supporting data are derived from *in vitro* or *ex vivo* studies of chlorpyrifos, comparisons to other agents acting on implicated systems, and basic neurobiological research. Some of the more promising mechanisms (described in detail in the 2012 SAP) represent events at different levels of biological organization, including molecular (binding to muscarinic receptors, endocannabinoid enzymes, tubulin, morphogenic site of AChE), cellular (reactive oxygen species, neurite growth, intracellular signaling) and functional (serotonergic tone, axonal transport). In some cases these effects occur at chlorpyrifos oxon exposure levels at or below those that result in cholinesterase inhibition in the same test system, or with the parent chlorpyrifos that has no esterase inhibitory properties. The 2008 and 2012 SAP concurred with the agency about the lack of definable AOPs, and the 2014 literature review showed no substantive changes in this conclusion. These experimental mechanistic studies do not provide adequate linkages from the initiating to subsequent events leading to alterations at the molecular and cellular level leading to neurobehavioral effects; this significantly limits these studies for quantitative use in risk assessment. Thus, while there is good evidence that neurodevelopmental effects may not be solely a function of AChE inhibition, there are no accepted alternative AOPs and the use of these studies for the assessment of chlorpyrifos and/or its oxon is challenging.

Numerous studies have evaluated chlorpyrifos and its oxon for biological activity against relevant targets and some include TCPy. For example, using PC12 cells, Das and Barone (1999) examined the concentration-related effects of chlorpyrifos, chlorpyrifos oxon, and TCPy on both neurite outgrowth and cholinesterase inhibition. Exposure to chlorpyrifos for 24 hr inhibited neurite outgrowth at a concentration (3 uM) 10-fold below that which inhibited cholinesterase activity, while chlorpyrifos oxon inhibited both measures at equivalent concentrations (1 nM). TCPy, which is inactive against AChE, inhibited neurite growth at 5 uM. Similar studies of chlorpyrifos and its metabolites were performed in a series of experiments in the Lein laboratory using primary neuronal cultures derived directly from the mammalian nervous system. Using rat sympathetic neurons, Howard et al. (2005) showed that 24-hour exposure to chlorpyrifos and chlorpyrifos oxon decreased axonal outgrowth at concentrations (0.001 uM and 0.001 nM, respectively) well below the concentrations that inhibited AChE activity (1 uM and 1 nM). In the same study chlorpyrifos, chlorpyrifos oxon, and TCPy enhanced dendrite outgrowth.

The agency acknowledges the lack of clearly identifiable toxic moiety(ies) associated with neurodevelopmental effects. There are limited data on the biological activity of TCPy across multiple types of assays. Moreover, TCPy is not a specific biomarker to chlorpyrifos which makes interpretation challenging. Given the potential for the oxon to be present in chlorinated drinking water combined with its high potency, the agency developed an exploratory evaluation of using the oxon as the toxic moiety associated with neurodevelopmental outcomes. However, given the complete lack of measured oxon values in biomarker data and the limited oxon data in laboratory animals, there are limited data that validate the oxon predictions in the PBPK mode. As such, the agency has not pursued the oxon for purposes of the 2016 evaluation. Thus, the remainder of this document focuses on the parent active ingredient, chlorpyrifos, as the key moiety associated with neurodevelopmental outcomes. The majority of the experimental laboratory studies and epidemiology studies from CCCEH have evaluated chlorpyrifos and provide a robust database of studies associating chlorpyrifos with neurodevelopmental outcomes.

The agency notes that there are a number of known developmentally neurotoxic chemicals with well-established relationships between exposure and neurological disorders in humans for which a definitive mode of action has not been established: for example lead, methyl mercury, and ethanol (Alfonso-Loeches & Guerri, 2011; Castoldi *et al.*, 2008; Farina, *et al.*, 2011; Johansson *et al.*, 2007; Verstraeten, *et al.*, 2008). Even today, with thousands of published papers on these three, accepted, developmental neurotoxicants, no coherent adverse outcome pathway or pathways can be constructed, because there are a multitude of possible initiating toxic events and cellular responses put forth, and they are not positively connected with one another. The only adverse outcome pathway available for any form of neurotoxicity has been developed for domoic acid in adult animals (Watanabe *et al.*, 2011), which has a large amount of preexisting data and a molecular initiating event at a receptor (glutamate) with known consequences to its overactivation in the adult nervous system. As such, the lack of AOP for neurodevelopmental outcomes mediated by chlorpyrifos does not preclude the agency from using this health outcome for human health risk assessment.

A.3.1.2 Studies on Laboratory Animals

There are an ever-increasing number of experimental studies that report a range of neurobehavioral changes in rats and mice following developmental exposure to chlorpyrifos. The agency has conducted a systematic review of these studies (USEPA, 2012, 2014, 2015). The agency has focused on studies where exposures occurred during gestation and/or postnatally, and testing took place after weaning and after chlorpyrifos exposure had ended; there is a presumption that these effects are permanent. There are inconsistencies in effects in relation to functional domains, gender-specificity, and relation to dose and/or AChE inhibition. Numerous differences in experimental design, dosing regimens, and test methodology probably influence the variability in outcomes observed across studies. In some cases, less-than-optimal procedures

and statistical analyses serve to lower confidence in the data, but at the same time the data are more convincing given the sheer number of studies reporting adverse outcomes.

Overall, some generalities emerge that support conclusions of broad changes in neurological function. Changes in various aspects of cognitive tests indicate perturbations of learning and/or memory, mostly reporting deficits even though in a few cases there is improved function. Alterations in anxiety and depressive behaviors, and social interactions, sometimes differed in direction of change, but are still suggestive of impacts on normal neuronal processing. Activity measures provide results as varied as the different measures of assessment. Obvious species differences have not emerged, and effective doses are similar (1–6 mg/kg/d) in rats and mice. Dose-response is not always evident, since many studies only use one dose, and of those using two or more doses, there is not always a monotonic response. Taken together, these data provide evidence for global alterations in neurobehavioral function rather than a specific profile of effects. This broad range of neurological effects does not aid in the development of a specific AOP (section A.4.1.1), and while there are numerous mechanistic studies that have proposed a number of plausible AOPs, none have been experimentally verified.

A.3.1.3 EPA Evaluation of Epidemiology Studies on Mothers & Children

A.3.1.3.1 Summary of Study Design and Scope of Evaluation

In April 2012, EPA presented to the FIFRA Scientific Advisory Panel (SAP) its review and assessment of several epidemiological investigations of the potential adverse neurodevelopmental outcomes of *in utero* and early life exposure to chlorpyrifos. In this effort, EPA limited its review to studies conducted within three major US based prospective birth cohort studies: 1) Columbia Center for Children's Environmental Health Mothers and Newborn Study, referred to in this document as "CCCEH" 2) Mount Sinai Inner-City Toxicants, Child Growth and Development Study, or the "Mount Sinai Study/Cohort;" and 3) Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley, or "CHAMACOS Study/ Cohort." The conclusion of EPA's evaluation, supported by the FIFRA SAP (2008, 2012), was that "chlorpyrifos likely played a role in the neurodevelopmental outcomes observed in these studies."

In the 2014 chlorpyrifos revised HHRA, EPA included epidemiologic research results from three prospective birth cohort studies. Importantly, each of these cohorts evaluated the association between prenatal chlorpyrifos or OP exposure with adverse neurodevelopmental outcomes in children through age 7 years. In addition, more recent studies from Mt. Sinai and CCCEH have evaluated these associations through age 9 years and 11 years, respectively (Furlong et al, 2014; Rauh *et al.*, 2015). EPA is not aware of any studies from the CHAMACOS cohort that have evaluated these associations in children older than 7 years old. These studies reflect different types of exposed groups in the total population which strengthens the weight of the evidence considerations regarding this stream of information. The CCCEH Mother's and Newborn study

and the Mt. Sinai Child Growth and Development study participants were likely exposed to OPs through the diet and through residential use of the pesticide for indoor pest control which was an allowable use for chlorpyrifos at the time the research was conducted. Additionally, residential exposure to OPs may also occurred via the oral route through ingesting residues from hand-to-mouth contact with in-home surfaces, as well as possible dermal or inhalation exposure through contact with treated areas in the home environment (Berkowitz *et al.*, 2003; Whyatt *et al.*, 2003, 2007, 2009).

In contrast, CHAMACOS cohort participants were employed as farm laborers or were residing in homes with farm laborers. The CHAMACOS study participants likely experienced exposure to OPs through the diet and from occupational exposure (primarily inhalation and dermal routes), as well as probable indirect take-home exposures (the "tracking in" of pesticide residues through shoes and clothing, augmented by poor hygiene practices) (Bradman *et al.*, 2007). In each of the three US children's health cohorts, the biological measurements in these cohorts were comparable to the general population NHANES. EPA has considered the strengths and limitations of these studies, and believes that random or systematic errors in the design, conduct or analysis of these studies were unlikely to fully explain the observed positive associations between *in utero* OP exposure and adverse neurodevelopmental effects observed at birth and through childhood (age 11 years). EPA believes these are strong studies which support a conclusion that OPs likely played a role in these outcomes.

In the 2015 updated literature review, the agency conducted a systematic review expanding the scope of the 2012/2014 review focused on US cohort studies with particular emphasis on chlorpyrifos. The expanded 2015 review includes consideration of the epidemiological data on any OP pesticide, study designs beyond prospective cohort studies, and non-U.S. based studies. The updated literature review identified seven studies which were relevant (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Shelton *et al.*, 2014). These seven studies have been evaluated in context with studies from the 2012/2014 chlorpyrifos review (USEPA, 2014a). This updated review did not change these conclusions, namely that OPs likely played a role in these outcomes reported in the epidemiology studies.

The CCCEH study measured parent chlorpyrifos in cord blood, and other indicators (*e.g.*, air sampling, behavioral information), as etiologic measures of exposure, while the other two birth cohorts measured non-specific urinary metabolites of chlorpyrifos and other OPs (TCPy, dialkyl phosphate metabolites) in the mothers to estimate pesticide exposure. Therefore, EPA considers the CCCEH study research results as most relevant to the chlorpyrifos HHRA; the other two cohorts provide important supporting information.

The biomarker data from the CCCEH studies are supported by the agency's 2014 dose reconstruction analysis using the PBPK-PD model. Following the recommendation of the

FIFRA SAP (2012), the agency conducted a dose reconstruction analysis of residential uses available prior to 2000 for pregnant women and young children inside the home (Appendix 2; USEPA, 2014a). Based on the output from the PBPK-PD model, for the highest exposure scenario considered (i.e., indoor broadcast use of a 1% chlorpyrifos formulation), <1% RBC AChE inhibition in pregnant women would be expected. While uncertainty exists as to actual OP exposure at (unknown) critical windows of exposure, EPA believes it is unlikely individuals in the epidemiology studies experienced RBC AChE inhibition.

Within the CCCEH epidemiology studies, the relationship in time between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes is concordant. The time period under study within the CCCEH study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, and researchers were able to show the change in exposure before (high use period) and after (low/no use period) the period of removal of chlorpyrifos products from the residential marketplace. Moreover, prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only 16% of the measured values were greater than the level of detection (LOD); there was only one child born in the time period subsequent to the voluntary cancellation of chlorpyrifos in the residential marketplace for whom the cord blood chlorpyrifos level was in the upper-tertile of pre-cancellation exposure levels. The significantly reduced proportion of measured values greater than the LOD as well as the observation of an absence of an association between prenatal chlorpyrifos exposure among infants born after the voluntary cancellation of chlorpyrifos and neurodevelopmental effects support the hypothesis that chlorpyrifos is related to these outcomes. However, as noted by study authors, EPA and the FIFRA SAP (2012), this could also be due to inadequate sample size to detect a small-to-modest effect among the group of infants born after the voluntary cancellation. It is notable that epidemiology studies from other research groups have not included analyses across different years of exposure.

Several studies have been excluded from further evaluation in this analysis. EPA considered studies available for the 2014 updated HHRA, the 2015 updated literature review (USEPA, 2015), and studies published since the 2015 updated literature review. As summarized below, excluded studies included those related to birth outcomes, autonomic nervous system impacts, and those with inadequate exposure assessment methods.

There has been inconsistent evidence of OP exposure and association with adverse birth outcomes/fetal growth outcomes. Authors with CCCEH observed evidence of an inverse association, *i.e.*, increasing cord blood chlorpyrifos was associated with decreased measures of birth weight and length, while authors with the Mt. Sinai and CHAMACOS cohorts reported either no association, or evidence of a *positive* relationship, respectively (Berkowitz *et al.*, 2004; Eskenazi *et al.*, 2004; Whyatt *et al.*, 2004). More recent studies evaluated in 2015 (USEPA, 2015) also documented inconsistent evidence of OP exposure and association with adverse birth

outcomes/fetal growth (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). Inconsistent results may be due to differences across study groups in exposure profiles as well as dissimilar methods of prenatal OP exposure assessment (Needham, 2005). Given the lack of consistency among cohorts for the fetal growth metrics, the proposed link between fetal growth and OP exposure is tenuous. Therefore, consistent with previous SAP evaluations for chlorpyrifos (FIFRA SAP 2008; FIFRA SAP 2012), EPA is focusing this analysis on neurodevelopmental outcomes. Although the agency is not evaluating these birth outcome studies further at this time, the agency will continue to monitor the scientific literature for advances in this line of research.

In the CHAMACOS study, impacts of prenatal DAP exposure and autonomic stability were also assessed (Young *et al.*, 2005). When all children in the study are considered together, there were no statistically significant associations for prenatal DAP exposure and autonomic stability. Stratifying the children by age, children ≤3 days had a statistically significant association between total DEAP exposure and autonomic stability, but no association for total DMAP and total DAP exposure. Children older than 3 days had no statistically significant associations with prenatal DAP exposure and autonomic stability. In addition, another study on the CHAMACOS birth cohort (Quirós-Alcalá *et al.*, 2011) assessed the association between DAPs and autonomic nervous system (ANS) outcomes at ages 6 months, 1 year, 3.5 years, and 5 years. The ANS outcomes assessed included heart rate and respiratory sinus arrhythmia. Overall, while there was some evidence of ANS dysregulation for infants at 6 months, these results were not consistently observed for the other assessed child (1 year, 3.5 years, and 5 years) and maternal OP exposures. There is not a body of literature to compare these results against, making it difficult to put them into context. Furthermore, these CHAMACOS studies did not focus on neurodevelopmental outcomes, which is the focus of this analysis. Therefore, due to the outcomes assessed and the lack of understanding of these findings, these studies are considered to be outside of the scope of this assessment and are not discussed further here. Although the agency is not evaluating this study data further at this time, the agency will continue to monitor the scientific literature for advances in this line of research.

The focus of this assessment is on the neurodevelopmental outcomes from exposures to low levels of chlorpyrifos (i.e. below exposures which would result in 10% or more AChE inhibition). Three studies conducted in Ecuador focused on child AChE inhibition and the potential association of AChE inhibition with other measures including parental occupation (Suarez-Lopez *et al.*, 2012), as well as clinical autonomic nervous system (ANS) outcomes such as blood pressure and heart rate (Suarez-Lopez *et al.*, 2013a), and neurodevelopmental outcomes (Suarez-Lopez *et al.*, 2013). The range of AChE activity levels are lower in the first tertile (range of 1.44 to 2.93 U/mL) compared to the third tertile (range from 3.33 to 4.69 U/mL). Therefore, due to the outcomes assessed and the potentially toxic cholinergic effects that were associated with these outcomes, these studies are not considered to be relevant to this assessment and are not discussed further here. Although the agency is not evaluating these study data

further at this time, the agency will continue to monitor the scientific literature for advances in this line of research.

Finally, six studies identified in the 2015 updated literature review (USEPA, 2015) did not have sufficient exposure assessment methods to determine whether exposure to OPs (including chlorpyrifos) actually occurred. These studies were conducted on study populations in Spain (Llop *et al.*, 2013), Ecuador (Handal *et al.*, 2007; 2007b; 2008), Denmark (Andersen *et al.*, 2015), and France (Petit *et al.*, 2010). In these studies, participants were considered exposed or unexposed to pesticides based on non-specific exposure measures, such as self-reported occupational exposure, home pesticide spraying, and proportion of municipality devoted to agricultural activity. For all of these proxy exposure assessments, the pesticides used may have included not only OPs, but also pyrethroids, fungicides, and growth regulators. Given the uncertainty about whether OP exposure actually occurred in these studies and whether observed outcomes are associated with OP exposure or with other pesticides, these studies were excluded from further analysis.

A.3.1.3.2 Summary of Findings in Epidemiology Studies

For the purposes of providing background and context, the study findings and study design used in these three cohort studies (CCCEH, CHAMACOS, and Mt. Sinai) and in the expanded 2015 literature review are summarized below. These three cohort studies each enrolled pregnant women during roughly the same time period, measured both environmental exposure to the pesticide during pregnancy and also measured biomarkers representing internal dose during pregnancy and at delivery, and prospectively assessed associations in their newborns and young children through age 7 years. In addition, more recent studies from Mt. Sinai and CCCEH have evaluated these associations through approximately age 9 years and 11 years, respectively (Furlong *et al.*, 2014; Rauh *et al.*, 2015). EPA is not aware of any studies from the CHAMACOS cohort that have evaluated these associations in children older than 7 years old.

Each study includes several hundred (approximately 100-400) mother-infant pairs; these sample sizes are sufficient to perform statistically valid analyses. Investigators from each study cohort utilized a similarly strong study design (prospective birth cohort); measured pesticide exposure using several different methods including environmental indicators as well as specific and non-specific biomarkers of OPs; ascertained developmental outcomes using validated assessment tools well-established in both clinical and research settings; and, measured, analyzed, selected and statistically adjusted for potential confounding variables including socio-economic status and other environmental exposures using reasonable and appropriate methods. Limitations exist as well. These studies utilized a one-time measure (or the average of two measures) of chlorpyrifos or OP exposure to assess prenatal pesticide exposure throughout the gestational period, were unable to assess the influence of mixtures (co-occurring exposures in the relevant biological time

window), and reflect a small sample size to fully evaluate the effect of more than one simultaneous exposure on neurodevelopment, *i.e.*, evidence of effect modification.

The OP exposure being assessed in many of these studies used concentrations of urinary dialkyl phosphate metabolites (DAPs) as the urinary biomarker. Total DAPs is a non-specific measure of OP exposure and is the sum of six separate molecules — three dimethyl alkylphosphate (DMAP) molecules of dimethylphosphate, dimethylthiophosphate, and dimethyldithiophosphate (DMP, DMTP, and DMDTP), and three diethyl alkylphosphate (DEAP) molecules of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate (DEP, DETP, and DEDTP). Each metabolite is a breakdown product from multiple OPs (CDC, 2008).[56] Specifically, DMP, DMTP, and DMDTP are associated with 18, 13, and 5 OPs, respectively; whereas DEP, DETP, and DEDTP are associated with 10, 10, and 4 OPs, respectively. Thus, in using urinary DAPs alone as an exposure measure, it is not possible to separate the exposure and associated effects for single, specific OPs.

DAPs can be found directly on food following OP applications (Zhang *et al.*, 2008; Chen *et al.*, 2012). Specifically, studies have shown that DAPs may form as environmental degradates from abiotic hydrolysis, photolysis, and plant metabolism (Zhang *et al.*, 2008; Chen *et al.*, 2012; Racke *et al.*, 1994). Furthermore, since these DAPs are excreted more rapidly and extensively than the parent OPs (Zhang *et al.*, 2008; Forsberg *et al.*, 2011), direct exposure to DAPs may lead to an overestimate of OP exposure when using urinary DAPs as a biomarker of OP exposure. The agency recognizes that this is a source of uncertainty when using DAPs for assessing OP exposure and will continue to monitor this issue in future assessments.

CCCEH studies have largely used chlorpyrifos measured in cord blood as the specific biomarker (*e.g.*, Lovasi *et al.*, 2011; Whyatt *et al.*, 2004; Rauh *et al.*, 2011). However, a few studies including CCEH measured urinary 3,5,6-trichloro-2-pyridinol (TCPy) (e.g., Fortenberry *et al.*, 2014; Eskenazi *et al.*, 2007; Whyatt *et al.*, 2009). This metabolite must be interpreted with caution. TCPy is also the primary environmental degradate of chlorpyrifos, chlorpyrifos-methyl, and triclopyr; thus exposure can be found directly on food treated with these pesticides.

The CHARGE study (Shelton *et al.*, 2015) did not measure biomarkers but instead used geospatial analysis to focus on the residential proximity to OP exposure using data from the California Department of Pesticide Regulation, with five OPs accounting for a total of 73% of the pesticide applied near residential settings (chlorpyrifos, acephate, diazinon, bensulide, and dimethoate).

As noted, two major uncertainties in environmental epidemiology studies are the accurate and reliable measurement of exposure and potential confounding variables such as the influence of mixtures. The researchers with each of the three cohorts have provided supplemental

[56] http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l26opd_c_met_organophosphorus_pesticides.pdf

methodological research to address these areas to the extent possible. Across the three children's health cohorts, study authors measured biomarkers of OP exposure. There is uncertainty as to the extent measurement of non-specific metabolites of OP or chlorpyrifos accurately reflects OP exposure; CCCEH and Mt. Sinai studies do not estimate post-natal exposure to chlorpyrifos among child participants, therefore the influence of early life and childhood OP exposure is unaccounted for in these analyses. The CHAMACOS cohort measured urinary levels of DAPs in young children and did not observe negative significant associations in relation to neurodevelopment from post-natal exposure (Eskenazi *et al.*, 2007). The CHAMACOS cohort investigators also measured AChE and butyl ChE as supplemental indicators of OP exposure.

Potential confounding bias is another major uncertainty within environmental epidemiology studies. Confounding variables, exposures related to OP exposure and neurodevelopmental outcomes, (*e.g.*, socioeconomic status or SES), may result in an incorrect epidemiological risk estimate. Across these cohort studies, investigators collected relevant information concerning demographic characteristics and other environmental exposures, and were, to the extent possible with the existing information, able to effectively control the influence of these other variables when estimating the association between prenatal chlorpyrifos and adverse neurodevelopmental outcomes. Control of these variables is important to reduce the chances of a false positive (or negative) study result. Overall, statistical analyses were judged to be appropriate and reasonable (not overly large number of statistical model variables) to the research question by EPA and expert Panel reviews (FIFRA SAP 2008 and 2012).

Researchers with both the Mt. Sinai and CHAMACOS cohorts evaluated neonatal neurological functioning in association with prenatal OP exposure; CCCEH did not conduct these measurements. To measure indices of abnormal neonatal behavior and/or neurological integrity authors used outcome measures derived from the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), a neurological assessment of 28 behavioral items and 18 primitive reflexes. This tool was administered to infants 2–5 days post-partum by trained neonatologists in the hospital setting using similar environmental conditions. The authors with both study groups observed an increased number of abnormal reflexes in relation to increasing measures of OP exposure (Engel *et al.*, 2007; Young *et al.*, 2005). Among the other 27 measures in the BNBAS, neither study group reported evidence of any other positive associations. The authors also observed evidence of potential effect modification by PON1 activity level in the relation between DAPs and neonatal neurodevelopment in which infants of mothers who are slower metabolizers have greater risk of abnormal reflexes (Young *et al.* 2005; Engel *et al.* 2007). However, EPA notes these studies are likely under-powered to make a statistically robust estimate of this statistical interaction. Similarly, a Chinese cohort study (Zhang *et al.*, 2014) reported statistically significant associations between total DEAPs, total DMAPs, and total DAPs from prenatal OP pesticide exposure and neonatal neurodevelopment assessed 3 days after birth. However, another cross-sectional Chinese study, Guodong *et al.* (2012), observed no association

with postnatal child urinary DAPs and a developmental quotient score for 23–25 month old children.

Researchers across the three children's health cohorts utilized the Bayley Scales of Infant Development II (BSID-II) to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) to assess neurodevelopment in early childhood. In the CCCEH Mothers and Newborn study, Rauh *et al.* (2006) investigated MDI and PDI at 12, 24, and 36 months of age. Children were categorized as having either high (>6.17pg/g) or low (≤6.17pg/g) prenatal chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics (Whyatt *et al.*, 2004). Authors reported that the difference in MDI scores was "marginally significant" (p=0.06) between the "high" and "low" exposed groups; the high exposed group scored an average of 3.3 points lower than the low exposed (Rauh *et al.*, 2006). Regarding the PDI score (motor skills), none of the 12 or 24 month PDI scores showed significant effects, but the 36 month score was significantly related to chlorpyrifos exposure. Researchers noted that the effects were most pronounced at the 36 month testing period. Within the 36 month testing period, the likelihood of highly exposed children developing mental delays were significantly greater (MDI: 2.4 times greater (95% CI: 1.12-5.08, p=0.02) and PDI: 4.9 times greater (95% CI: 1.78-13.72; p=0.002)) than those with lower prenatal exposure (Rauh *et al.*, 2006). Within the Mt. Sinai study, authors administered the BSID-II to participating children at 12 and 24 months and observed that prenatal total DAP metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanic children; however, these associations either attenuated or were non-existent at the 24-month visit (Engel *et al.*, 2011). In the CHAMACOS cohort, Eskenazi *et al.* (2007) observed that prenatal DAP levels were adversely associated with MDI, and at 24 months of age these associations reached statistical significance. In this study, neither prenatal DAPs nor maternal TCPy were associated with PDI (motor skills), nor did authors observe evidence of different risk by PON1 status (Eskenazi *et al.*, 2010).

Most recently, Engel *et al.* (2015) reports on the results of a pooled analysis from four cohorts (N=936) to evaluate the association between prenatal urinary DAPs and neurodevelopmental outcomes (MDI/PDI) at 24 months only. The four cohorts include CHAMACOS (N=377), HOME (N=265), Mt. Sinai (N=234), and CCCEH (N=60). The Cincinnati Children's HOME Study is a newer cohort which collected study data from mothers and infants in 2003 to 2006, and no separate studies have been published yet on OP exposure and associations with neurodevelopmental outcomes. It is noted that the CCCEH participants included in this analysis are from women enrolled in 2000 to 2001, a time period which is during the phase out of chlorpyrifos in residential settings. The results of this pooled study are relatively consistent with those seen in the individual cohorts at 24 months. After controlling for race/ethnicity, smoking, and drug use during pregnancy, a statistically significant association was observed in the pooled population between total DAPs exposure and MDI decrements, but not with PDI decrements. Consistent with the results from Eskenazi *et al.* (2007), the strongest evidence of an association

was observed for the CHAMACOS cohort, with statistically significant associations for both total DAPs and total DMAPs exposure and MDI decrements. No significant associations were seen within the Mt. Sinai and CCCEH cohorts, a result which is basically consistent with the previous observations at 24 months in these cohorts (Engel *et al.*, 2011; Rauh *et al.*, 2006). The study authors observed significant heterogeneity from combining the cohorts, especially with regards to race/ethnicity, and noted that impacts on specific subpopulations may be lost when looking at the pooled results.

With respect to the findings related to the autism spectrum, from CCCEH, Rauh *et al.* (2006) reported a large odds ratio for pervasive developmental disorder (PDD) (OR=5.39; 95% CI: 1.21–24.11) when comparing high to low chlorpyrifos exposure groups. As described above, among 7–9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness. However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEAP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMAP biomarker levels. In the CHAMACOS cohort, Eskenazi *et al.* (2010) reported non-significant, but suggestive, increased odds of PDD of 2.0 (0.8 to 5.1; p=0.14), whereas Eskenazi *et al.* (2007) reported a statistically significant association between total DAP exposure and increased odds of PDD. Additionally, Furlong *et al.* (2014) documented suggestive evidence of an association between total DEAP exposure and reciprocal social responsiveness among blacks and boys. Using a different exposure assessment method (geospatial analysis and residential proximity to total OP exposure), Shelton et al. (2014) also showed statistically significant associations between total OP exposure and ASD.

With respect to attention problems, Rauh *et al.* (2006) also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems (p=0.010), and in the more specific DSM-IV scale for ADHD problems (p=0.018). The CHAMACOS cohort also investigated attention problems in early childhood using three different assessment tools: maternal report of child behavior at 3.5 and 5 years of age; direct assessment of the child at 3.5 and 5 years; and by a psychometrician's report of the behavior of the child during testing at 5 years. In this study population, higher concentrations of OP metabolites in the urine of pregnant women were associated with increased odds of attention problems and poorer attention scores in their children at age 5 years (Eskenazi *et al.*, 2007). Additionally, in a Mexican cohort study, Fortenberry *et al.* (2014) found suggestive evidence of an association with TCPy and ADHD in boys. In a national cross-sectional study of Canadian children, using 2007–2009 data for children age 6–11 years (Oulhote and Bouchard, 2013), there were no overall statistically significant associations observed between child urinary DEAP, DMAP, or total DAP metabolite

levels and parentally reported behavioral problems. In contrast, Bouchard *et al.* (2010), looking at U.S. children age 8–15 years in the 2000–2004 National Health and Nutrition Examination Survey (NHANES), observed a positive association between attention and behavior problems and total DAPs and DMAPs, but not DEAPs. As part of their analysis, Oulhote and Bouchard (2013) noted that their outcome assessment for behavioral problems may not have been as sensitive as Bouchard *et al.* (2010), which may in part account for the difference in the observed results from these studies.

To measure intelligence among school aged children, authors from each of the three children's health cohorts used the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV). The instrument measures four areas of mental functioning: the Verbal Comprehension Index, the Perceptual Reasoning Index, the Working Memory Index, and the Processing Speed Index. A Full-Scale IQ score combines the four composite indices. WISC-IV scores are standardized against U.S. population-based norms for English and Spanish-speaking children. In the CCCEH Mothers and Newborn Study, Rauh *et al.* (2011) evaluated the relationship between prenatal chlorpyrifos exposure and neurodevelopment among 265 of the cohort participants who had reached the age of 7 years and had a complete set of data including prenatal maternal interview data, prenatal chlorpyrifos marker levels from maternal and/or cord blood samples at delivery, postnatal covariates, and neurodevelopmental outcome data (Rauh *et al.*, 2011). While models were developed using continuous measures of both prenatal chlorpyrifos exposure and Wechsler scores, for ease of interpretation, investigators reported that for each standard deviation increase in exposure (4.61 pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory. In the Mt. Sinai study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in Full Scale Intelligence Quotient (FSIQ), Perceptual Reasoning, and Working Memory between the ages of 6 and 9 years, and difference in intelligence measures by putative PON1 status were also noted (Engel *et al.*, 2011). Similarly, in the CHAMACOS cohort, Bouchard *et al.* (2011) observed evidence of an association between prenatal exposures to OPs as measured by urinary DAP (total DAP, DEP, and DMP) metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7. In this study, children in the highest quintile of maternal DAP concentrations had a statistically significant 7-point difference in IQ points compared with those in the lowest quintile.

To ascertain whether observed differences in neurodevelopment after prenatal chlorpyrifos exposure may be explained by differences in brain morphology between exposed groups, investigators compared MRI brain images between high and low chlorpyrifos exposed child study participants (Rauh *et al.*, 2012). Authors determined there were distinct morphological differences in brain areas associated with these neurodevelopmental outcomes. The pilot study included 40 child participants due to strict inclusion and exclusion criteria, and the high cost of performing the imaging studies on each child. EPA convened a Federal Panel of experts to

perform a written peer-review of this study.[57] The Federal Panel concurred with the authors' conclusions in general; however the Federal Panel also noted that significantly greater and more sophisticated MRI imaging studies would be needed to link the morphological changes indicated in this study with specific functional outcomes noted in the CCCEH IQ study. Therefore, while generally supportive of the epidemiologic findings, additional study is needed to make specific links with areas of brain development change.

Most recently, CCCEH study authors (Rauh *et al.*, 2015) evaluated the relationship between prenatal chlorpyrifos exposure and motor development/movement among 263 of the cohort participants who had reached the age of 11 years and had a complete set of data including prenatal maternal interview data, prenatal chlorpyrifos marker levels from maternal and/or cord blood samples at delivery, postnatal covariates, and motor development/movement outcome data (Rauh *et al.*, 2015). When comparing children in the upper quartile of exposure (>6.17 pg/g; N=43) to those in the lower quartiles, they observed statistically significant associations between prenatal chlorpyrifos exposure and mild to moderate tremor in the arm. These associations were observed even after controlling for potential confounding factors such as medication, sex, and ethnicity.

In sum, across these three children's environmental health studies (CCCEH, Mt. Sinai, and CHAMACOS) and in the seven studies identified in the 2015 literature review, authors consistently identified associations with neurodevelopmental outcomes in relation to OP exposure. There is evidence of delays in mental development in infants (24–36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to chlorpyrifos or OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2–4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures.

A.3.2 Summary of EPA's Weight of Evidence Analysis

In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of scientific evidence in the context of the understanding of the adverse outcome pathway (or mode of action (U.S. EPA, 2010). The draft framework was reviewed favorably by the SAP in 2010 (FIFRA SAP, 2010). OPP's draft framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework, which highlight the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek *et al.*, 2014). Consistent with recommendations by the NRC in its 2009 report on *Science and Decisions*, OPP's draft

[57] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170.

framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage starts with planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern and exposure pathways and duration along with key gaps in data or scientific information. Specific to chlorpyrifos, the 2008 and 2012 SAP reviews represent the problem formulation analyses for the weight-of-evidence (WOE) evaluation. The modified Bradford Hill Criteria (Hill, 1965) are used to evaluate the experimental support that establishes key events within a mode of action or an adverse outcome pathway, and explicitly considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight of evidence analysis. The full weight of evidence evaluation can be found in the 2014 human health risk assessment.

Dose-response relationships & temporal concordance.

Since the MOA(s)/AOP(s) is/are not established for neurodevelopmental outcomes, it is not possible to describe the concordance in key events or biological steps leading to neurodevelopmental outcomes. As such, the quantitative linkages between MIEs, intermediate steps, and ultimately the adverse outcome (*i.e.*, neurodevelopmental effects) cannot be determined. Experimental toxicology studies in rodents suggest that long-term effects from developmental chlorpyrifos exposure may occur. Due to the dose selections in most of these *in vivo* studies evaluating effects such as behavior and cognition, it is not known whether such adverse effects would be shown at doses lower than those which elicit 10% RBC AChE inhibition.

Within the epidemiology studies, the relationship in time between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes is concordant. Specifically, with regard to the children's environmental health epidemiology studies, each of the three study cohorts utilized a prospective birth cohort study design in which mothers were recruited into study prior to the birth of the infants and development and identification of adverse effects; therefore, it is known with certainty that exposure preceded effect. In addition, because the time period under study within these cohorts, and specifically the CCCEH study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, researchers were able to show the change in exposure before (high use period) and after (low/no use period) the period of removal of chlorpyrifos products from the residential marketplace. Moreover, prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only 16% of the measured values were greater than the LOD; there was only one child born in the time period subsequent to the voluntary cancellation of chlorpyrifos in the residential marketplace for whom the cord blood chlorpyrifos level was in the upper-tertile of pre-cancellation exposure levels. The significantly reduced proportion of measured values greater than the limit of detection as well as the

observation of an absence of an association between prenatal chlorpyrifos exposure among infants born after the voluntary cancellation of chlorpyrifos support the hypothesis that chlorpyrifos is related to these outcomes. However, as noted by study authors, EPA and the FIFRA SAP (2012), this could also be due to inadequate sample size to detect a small to modest effect among the group of infants born after the voluntary cancellation.

With respect to the timing of exposure, the cord blood and other (meconium) measures from the CCCEH study provide evidence that exposure did occur to the fetus during gestation. For these epidemiology studies, it is important to note that chlorpyrifos was only assessed directly in the CCCEH study (Rauh *et al.*, 2006; Rauh *et al.*, 2012), with the Mexican cohort study (Fortenberry *et al.*, 2014) assessing the chlorpyrifos metabolite TCPy, and the CHAMACOS cohort study (Eskenazi *et al.*, 2007) measuring both TCPy and DAPs. In contrast, all of the other epidemiology studies assessed only DAP exposure, and as noted previously these DAPs are metabolites of multiple OPs including chlorpyrifos.

Exposures measured in the range reported in the epidemiology studies (pg/g plasma) are low enough that is unlikely to result in AChE inhibition. The urinary TCPy concentrations among mothers were comparable to the general population levels measured in NHANES. This biomarker data from the CCCEH studies are supported by the agency's dose reconstruction analysis using the PBPK-PD model. In accordance with the recommendation of the FIFRA SAP (2012), the agency conducted a 2014 dose reconstruction analysis of residential uses available prior to 2000 for pregnant women and young children inside the home. Based on the output from the PBPK-PD model, for the highest exposure considered (*i.e.*, indoor broadcast use of a 1% chlorpyrifos formulation), <10% RBC AChE inhibition in pregnant women and young children would be expected from residential uses. It is noteworthy that all estimates of exposure based on conservative assumptions lead to predicted AChE inhibition levels <10%.

Strength, Consistency, and Specificity.

EPA notes consistencies across the databases of animal toxicology and epidemiology studies. Considering the toxicological and epidemiological data in the context of three major neurodevelopmental domains (specifically, cognition, motor control, and social behavior), insights can be gained. For example, chlorpyrifos studies in rats and/or mice have reported impaired cognition (spatial learning and working memory; *e.g.*, Icenogle *et al.*, 2004, Billauer-Haimovitch *et al.*, 2009); changes in locomotor activity levels (exploration, rearing; *e.g.*, Levin *et al.*, 2002; Ricceri *et al.*, 2003); and altered social interaction (aggression, maternal behavior; Venerosi *et al.*, 2006, 2010); and effects on brain morphometrics (MRID 44787301; Chen *et al.*, 2012). Similarly, epidemiologic investigations have reported effects on cognition (Bayley scale indices; Rauh *et al.*, 2006; Eskenazi *et al.*, 2007), abnormal motor development in neonates (reflexes, Brazelton score or similar measure; Young *et al.*, 2005; Engel *et al.* 2007; Zhang *et al.*, 2014), altered social development (*e.g.,* ADHD; Rauh *et al.*, 2006; Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014), and changes in MRI brain scans (Rauh *et al.*, 2012).

It is notable that the laboratory animal studies vary in experimental designs such as species, strain, gender, dosing regimens (age, routes, vehicle), and test parameters (age, protocol). Likewise, observational epidemiology studies vary by population characteristics (race/ethnicity, SES, and pesticide use/exposure profile), co-exposures (mix of chemicals, windows of exposure), and method of exposure and outcome assessment. Given the differences across laboratory animal and epidemiology studies, the qualitative similarity in research findings is striking.

In contrast, quantitatively, there are notable differences between animals and humans. Specifically, in animals, the doses most often used in the behavior studies (1 and 5 mg/kg/day) are sufficient to elicit approximately ≥10% brain inhibition and ≥30% in RBC inhibition , depending on the study design, age of the animal, and sampling time. Even in one study using a lower dose (0.36 mg/kg/d in feed), this level was sufficient to produce a great degree of maternal RBC AChE inhibition (Ohishi *et al*., 2013). There are essentially no animal studies that have tested at doses shown to not inhibit AChE under the exposure conditions. In the epidemiology studies, based on the comparisons with biomonitoring data and the results of the dose-reconstruction analysis, it is unlikely that RBC AChE would have been inhibited by any meaningful or measurable amount, if any at all, and most likely none in the brain (although the agency has not investigated the degree to which exposure to multiple AChE-inhibiting pesticides indoors simultaneously could impact this conclusion). This key difference in dose response between the experimental toxicology and epidemiology studies poses challenges in interpreting such data. There are a number of possible hypotheses such as: 1) limitations of experimental laboratory studies which have limited statistical power due to relatively small sample sizes; 2) humans display a broader array of behaviors and cognitive abilities than rats, thus limiting the sensitivity of the rat studies; and 3) in the epidemiology studies, the timing of chlorpyrifos application and blood collections are not coupled—thus higher levels of blood chlorpyrifos were likely missed (albeit the results of the dose reconstruction analysis reduce the likelihood of this hypothesis).

In making a weight-of-evidence analysis, it is important to consider the strength of the statistical measures of association between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes through childhood (epidemiology) and possibly into adulthood (animal studies). It is also important to consider the strength of the integrated qualitative and quantitative evidence, the consistency of the observed associations across epidemiology studies and considering both animal and human data that support the conclusion that chlorpyrifos plays a role in adverse neurodevelopmental outcomes. While it cannot be stated that chlorpyrifos alone is the sole contributor to the observed outcomes (specificity), since other environmental, demographic or psychosocial exposures may also play a part in these outcomes, this does not obviate the contribution of prenatal chlorpyrifos exposure in the development of adverse neurodevelopmental outcomes as echoed by the FIFRA SAP (2012).

The CCCEH study, which measures chlorpyrifos specifically, provides a number of notable associations. Regarding infant and toddler neurodevelopment, the CCCEH authors also reported statistically significant deficits of 6.5 points on the Bayley Psychomotor Development Index (PDI) at 3 years of age when comparing high to low exposure groups (Rauh *et al.*, 2006). Notably these decrements in PDI persist even after adjustment for group and individual level socioeconomic variables (Lovasi *et al.*, 2011). These investigators also observed increased odds of mental delay (OR=2.4; 95% CI: 1.1–5.1) and psychomotor delay (OR=4.9; 95% CI: 1.8–13.7) at age three when comparing high to low exposure groups (Rauh *et al.*, 2006). Rauh *et al.* (2006) also reported large odds ratios for attention disorders (OR=11.26; 95% CI: 1.79–70.99), ADHD (OR=6.50; 95% CI: 1.09–38.69), and PDD (OR=5.39; 95% CI: 1.21–24.11) when comparing high to low chlorpyrifos exposure groups (Rauh *et al.*, 2006). EPA notes that the magnitude of these results are so large that they are unlikely to be affected by residual confounding although limited sample sizes resulted in imprecise estimates.

Importantly, across the three children's environmental health birth cohorts, decrements in intelligence measures were identified in relation to increasing levels of prenatal chlorpyrifos exposure. Authors from the CCCEH cohort reported statistically significant decreases of 1.4% in full scale IQ and 2.8% in working memory among seven-year olds for each standard deviation increase in chlorpyrifos exposure (Rauh *et al.*, 2011). These results persist even when performing sensitivity analyses including only those with detectable chlorpyrifos levels. In addition, no evidence was provided of mediation by child behavior on the measure of working memory instrument.

Biological plausibility and coherence. The 2012 SAP noted "Questions about biologic plausibility due to lack of clarity on mechanism of action, particularly at the low exposure levels seen in the cohorts and the mixed results of animal studies showing neurodevelopmental effects." EPA's Cancer Guidelines (2005) includes guidance which are also applicable to this current evaluation of chlorpyrifos. The Guidelines indicate:

"evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies. [p. 39]."

The Cancer Guidelines further state that **"**lack of mechanistic data, however, is not a reason to reject causality [p. 41]."

At this time, a MOA(s)/AOP(s) has/have not been established for neurodevelopmental outcomes. This growing body of literature does demonstrate, however, that chlorpyrifos and/or its oxon are biologically active on a number of processes that affect the developing brain. Moreover, there is

a large body of *in vivo* laboratory studies which show long-term behavioral effects from early life exposure. EPA considers the results of the toxicological studies relevant to the human population, as qualitatively supported by the results of epidemiology studies. The lack of established MOA/AOP pathway does not undermine or reduce the confidence in the findings of the epidemiology studies. The CCCEH data are not considered in isolation, but rather are strengthened when considered in concert with the results from the other two cohort studies, as noted by the FIFRA SAP (July 2012). As noted above, the CHAMACOS and Mt. Sinai cohorts that measured neurological effects at birth (the Brazelton index), observed an association with DAPs exposure (Engel *et al.*, 2007; Young *et al.*, 2005). Similarly, while not consistent by age at time of testing (ranging from 6 month to 36 months across the three cohorts), each cohort reported evidence of impaired mental and psychomotor development. Attentional problems and ADHD were reported by both Mt. Sinai and CHAMACOS investigators. Finally, each of the three cohort study authors observed an inverse relation between the respective prenatal measures of chlorpyrifos or DAPs and intelligence measures at age 7 years.

Overall, the results from the seven studies identified in the 2015 literature review (USEPA, 2015), further strengthen the observations from the CCCEH study and the other two children cohort studies. Specifically, statistically significant associations were observed for: prenatal DAP exposure and neonatal development (Zhang *et al.*, 2014); total OP exposure and autism spectrum disorders (Shelton *et al.*, 2014); and a positive association between attention and behavior problems and total DAPs and DMAPs, but not DEAPs (Bouchard *et al.*, 2010). Suggestive evidence of associations were observed for: TCPy and ADHD in boys (Fortenberry *et al.*, 2014); and total DEAP exposure and reciprocal social responsiveness among blacks and boys (Furlong *et al.*, 2014). No associations were observed in two cross-sectional studies for: postnatal child urinary DEAP, DMAP, or total DAP metabolite levels and parentally reported behavioral problems in Canadian children (Oulhote and Bouchard, 2013); and for postnatal child urinary DAPs and a developmental quotient score for 23–25 month old children from China (Guodong *et al.*, 2012). It is noted that with the exception of Fortenberry *et al.* (2014), which measured TCPy exposure, these seven studies did not assess chlorpyrifos exposure directly and instead measured DAPs exposure.

Although uncertainties remain as articulated above, these uncertainties are diminished in the context of the qualitative similarity between the databases, and the concern for long-term neurodevelopmental effects as a result of prenatal, perinatal and possibly early life exposure.

Appendix 4.0: Background & Summary of the Chlorpyrifos PBPK Model

[Note: For the 2016 analysis, the agency is only using the PK portion of the PBPK model since the PD portion applies only to AChE inhibition.]

As described in detail in EPA's 2006 document entitled, "*Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment,"* physiologically based pharmacokinetic (PBPK) modelling is a scientifically sound and robust approach to estimating the internal dose of a chemical at a target site and as a means to evaluate and describe the uncertainty in risk assessments. PBPK models consist of a series of mathematical representations of biological tissues and physiological processes in the body that simulate the absorption, distribution, metabolism, and excretion (ADME) of chemicals that enter the body. Examples of PBPK model applications in risk assessments include interspecies extrapolation, intra-species extrapolation, route-to-route extrapolation, estimation of response from varying exposure conditions, and high-to-low dose extrapolation. PBPK models can be used in conjunction with an exposure assessment to improve the quantitative characterization of the dose-response relationship and the overall risk assessment. These models can also be used to evaluate the relationship between an applied dose and biomonitoring data.

Although the model includes the description of AChE inhibition, *in the 2016 analysis, the agency is only using the PK portion of the model*. The PBPK-PD model for chlorpyrifos that was originally developed by Timchalk and coworkers in 2002 (Timchalk *et al*., 2002a, b) has been refined over the years as more data has become available (Busby-Hjerpe *et al*., 2010; Cole *et al*., 2005; Garabrant *et al*., 2009; Lee *et al*., 2009; Lowe *et al*., 2009; Lu *et al*., 2010; Marty *et al*., 2007; Timchalk and Poet, 2008; Timchalk *et al*., 2005; Timchalk *et al*., 2006). The model will not be described in detail here as it is described in numerous publications, including a report reviewed by the FIFRA SAP in 2011; summary information is provided here. All model code for the PBPK-PD model are provided in the public docket for the chlorpyrifos risk assessment and for the 2016 SAP.

A.4.1 Introduction to the Physiologically-Based Pharmcokinetic/Dynamic Model

Evaluation of PBPK-PD models intended for risk assessments includes a review of the model purpose, model structure, mathematical representation, parameter estimation (calibration), and computer implementation (USEPA, 2006b). Developers of the chlorpyrifos PBPK-PD model sponsored a third-party quality assurance assessment to verify model parameter values and their respective sources. The agency has conducted a review of this third-party assessment by randomly checking a subset of the values and sources in the model parameters (USEPA, 2014d) and has continued to critically evaluate the code and its output. The agency also conducted a mass balance analysis. Minor inconsistencies were identified, and developers of the chlorpyrifos

PBPK-PD model have since made the corrections, or provided additional references to justify their parameterization choices. Recently, the agency performed additional evaluation of the code for dermal exposure; this evaluation did not change any aspects of the model code but supported agency confidence in the predictions (MRID 49830302). The mathematical equations in this model are adequate to reasonably predict both blood/urine dosimetry and ChE inhibition in two controlled, deliberate oral human dosing studies (Nolan *et al.*, 1984; Kisicki *et al.*, 1999) and a dermal human study (Nolan *et al.*, 1984)[58]. The PBPK-PD model predictions for rats inhaled chlorpyrifos compared well with animal data (Hotchkiss *et al.*, 2013) with respect to chlorpyrifos, oxon, and TCPy concentrations in plasma, and ChE in plasma, RBC and brain (Poet *et al.*, 2014). Significant improvements have been made to the PBPK-PD model in response to the 2008, 2011, and 2012 SAPs, the agency, and peer reviewers from academic journals in addition to the input of new data. The agency believes that the model is sufficiently robust for use in human health risk assessment.

Age-specific parameters are incorporated allowing for lifestage-specific evaluations from infancy through adulthood of chlorpyrifos, its oxon, and TCPy levels in various tissues, such as plasma and urine. The model can be run in two modes: deterministic and variation. In the deterministic mode, the output accounts for human specific metabolism and physiology thus obviating the need for the inter-species extrapolation factor for all for all age groups. In variation mode, distributions for16 parameters, which are critical for determining human variations in RBC AChE inhibition, are incorporated and thus the output accounts for intra-species extrapolation for infants, toddler, youths, and non-pregnant adults. For this 2016 issue paper, the focus is on deterministic evaluation; no probabilistic PBPK runs are provided in this issue paper.

The current model being used by the agency for the chlorpyrifos risk assessment does not include gestation or lactational exposure. With respect to lactational exposure, the agency is aware of breast milk pilot monitoring data from the Pilot study for the National Children's Study (NCS) Supplemental Methodological Studies (SMS) that can be used to evaluate infant exposure to chlorpyrifos through breastfeeding using. Although it would be useful to make predictions of breast milk exposures, since there are some (albeit limited) breast milk monitoring data from contemporary exposures, the lack of a lactational PBPK model does not introduce substantial uncertainty in the risk assessment.

In April 2015, DAS modified the multi-route PBPK/PD model for chlorpyrifos to include additional code to describe physiological changes for women during pregnancy. The gestational

[58] Two human deliberate dosing studies (Nolan *et al.* 1982; Kisicki *et al.*, 1999) are available which have been reviewed by EPA's Human Studies Review Board; http://www.epa.gov/osa/hsrb/files/june2009finalreport92609.pdf; http://www.epa.gov/osa/hsrb/files/meeting-materials/apr-13-14-2011/appendix1.pdf

component of the model includes the following modifications: (1) placenta, uterine, and fetal compartments, all of which grow over the course of pregnancy; (2) pregnancy-specific changes in the fat, rapidly-perfused, and slowly-perfused compartments; (3) pregnancy-specific changes in blood composition resulting in increased blood volume and decreased hematocrit; and (4) pregnancy-specific changes in metabolism, both CYP450s and PON1 enzymes based on published studies. No changes were made to the PD model since there are no data available to suggest cholinesterase changes during pregnancy. The growth of fetus, uterus, and placenta predicted by the model agreed with empirical data as were the changes in tissue and blood volume (Abduljalil, K., *et al*., 2012). While the modified model reasonably simulate the physiological changes during pregnancy, the model's predictive ability to simulate internal dosimetry of chlorpyrifos cannot be properly evaluated since there were no chlorpyrifos-specific pharmacokinetic data available during pregnancy. *As such, the agency cannot evaluate its predictive capacity and thus, the pregnancy model will not be used for risk assessment at this time.*

The agency does note, however, that the pregnancy model was built based on the best knowledge available and some preliminary simulations using this model suggested that when accounting for variability in physiology, In addition, an initial testing of the pregnancy version of the PBPK/PD model showed that, given the same oral dose, blood concentrations of chlorpyrifos in women in the third trimester were slightly lower than those in non-pregnant women (Appendix 6). While some parameter changes during pregnancy contributed to more chlorpyrifos in blood (e.g., smaller fat:blood partition during pregnancy) and other changes contributed to less chlorpyrifos in blood (e.g., increased tissue volumes for more storage), the combined effects from these multiple physiological changes during pregnancy resulted in slightly lower blood concentrations of chlorpyrifos during pregnancy. Similarly, RBC AChE inhibition in pregnant women at a given dose was comparable to non-pregnant women.

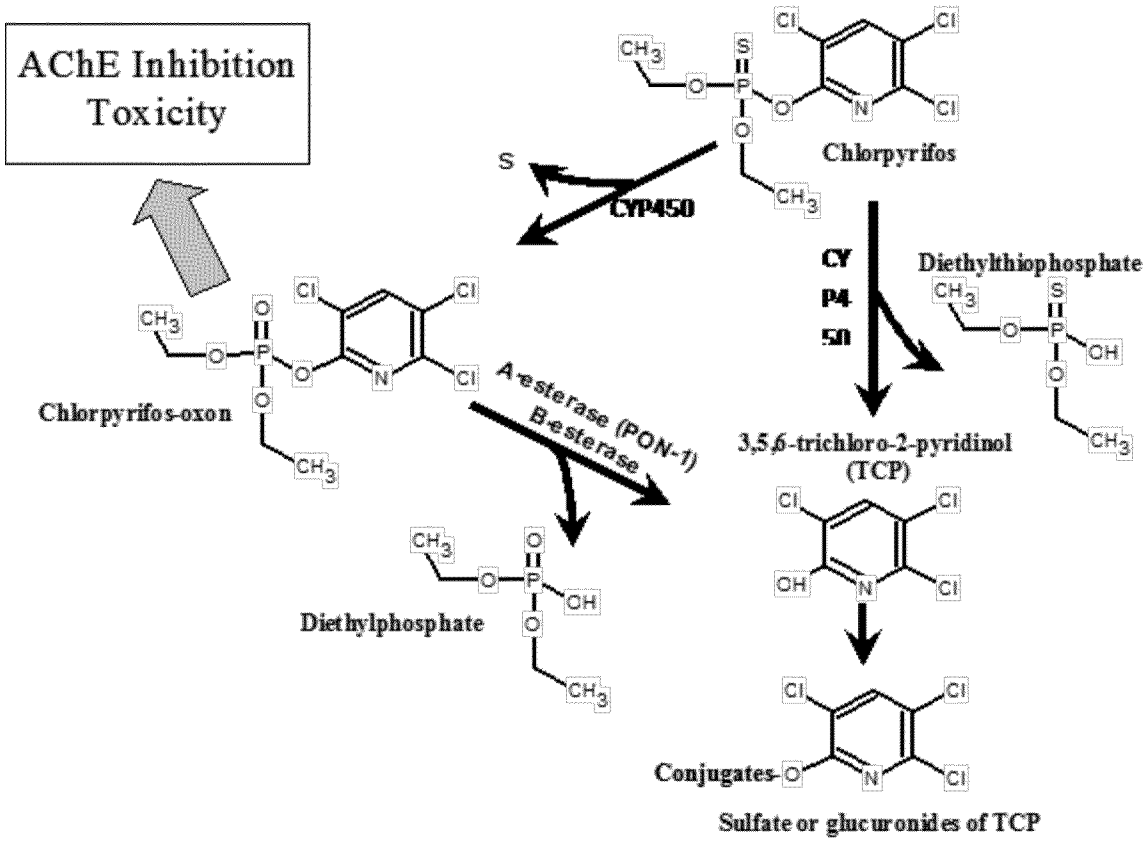
In addition, data from CCCEH suggest that maternal blood is a reasonable surrogate for cord blood. Whyatt *et al*. (2009) have shown that levels of chlorpyrifos in maternal blood and umbilical cord blood levels were highly correlated ($r = 0.9$, $p<0.001$, $n = 64$) with very similar values reported at the 90th (maternal and cord levels of 1.5 pg/g and 2.3 pg/g, respectively) and 95th percentiles (maternal and cord levels of 2.5 pg/g) suggesting that tracking the blood concentrations of the mother is a reasonable surrogate for the fetus. In a second paper, Whyatt *et al*. (2003) showed that chlorpyrifos in maternal blood and umbilical cord blood levels were highly correlated ($r = 0.76$, $p<0.001$, $n=180$ mother-child pairs. Although the agency would prefer to have a robust gestational PBPK model parameterized with chlorpyrifos data, the lack of such model robust does not add major uncertainty into the 2016 analysis.

A.4.2 Summary of Metabolic Profile

The metabolism and pharmacokinetic (PK) profiles of chlorpyrifos and its oxon have been extensively studied in *in vitro* systems, *in vivo* laboratory animals, as well as humans. This large body of PK information is used in the PBPK-PD model. Only summary information is provided here. Chlorpyrifos undergoes metabolic transformations mainly by the liver microsomal enzymes (*i.e.*, cytochrome P450s). The initial metabolic action of chlorpyrifos is desulfuration, resulting in bioactivation of the parent compound to the more toxic and potent AChE inhibitor, the oxon form. However, the oxon is unstable and is rapidly deactivated through hydrolytic cleavage by a process called dearylation releasing 3,5,6-trichloro-2-pyridinol (TCPy). Simultaneous with the desulfuration process, dearylation acts on both the parent chlorpyrifos as well as on the oxon metabolite leading to the release of TCPy. TCPy is further conjugated to form glycine or glucuronide conjugates and eliminated into the urine. TCPy is the major excreted metabolite and used as the biomarker in PK, biomonitoring, and epidemiology studies. Diethylphosphate (DEP) is another metabolite often used in biomonitoring studies, but since it is produced by a number of OPs, DEP is not a specific marker for chlorpyrifos. An important aspect of the chlorpyrifos PBPK model is that chlorpyrifos and its oxon are approximately 98–99% protein bound in the blood.

There are several enzymes that have roles in the metabolism and toxicity of chlorpyrifos. In addition to inhibition of ChE, the oxon binds stoichometrically to B-esterases, the most important of which are butyrylcholinesterse (BuChE; abundant in blood, brain, and other tissues) and carboxylesterases (highest levels in liver). These B-esterases function as a scavenger, or "sink", of the oxon and may lessen its entry in the brain or peripheral targets to inhibit AChE. Another group of important enzymes in the detoxification of chlorpyrifos is the A-esterases; one such A-esterase is paraoxonase (*i.e.*, PON1). These esterases are calcium-activated enzymes that are distributed in various tissues including the liver, brain and blood. These enzymes act on the oxon by hydrolyzing it before reaching its target AChE enzyme. The cytochrome P450 family of microsomal enzymes (CYPs) is also responsible for its metabolic activation and deactivation of chlorpyrifos. Glutathione-dependent enzymes play an important role in the secondary metabolism of chlorpyrifos producing water soluble metabolites that are readily excreted into the urine.

Figure A.1. Major metabolic pathways of chlorpyrifos metabolism (Reproduced from Timchalk et al, 2002b)



A.4.3 Description & Structure of the Physiologically-Based Pharmcokinetic/Dynamic Model

The chlorpyrifos PBPK-PD model includes descriptions of the ADME of chlorpyrifos, and its metabolites, oxon and TCPy (Figure A.2). The PBPK-PD model contains descriptions of metabolism to account for chlorpyrifos, its oxon, and TCPy in liver, blood, brain, small intestine, lungs, diaphragm, and skin. Model parameterization was achieved most often by extrapolation from *in vitro* studies (animal and human tissues), in some incidences by extrapolation from rat *in vivo* studies, and, for TCPy pharmacokinetics, by fitting to human data. The PD portion of the model is an extension of the PBPK model and relates the prediction of the formation of oxon to activity changes in AChE, BuChE, and carboxylesterase in brain, diaphragm, liver, lungs, plasma, and RBC. Because age-specific parameters are incorporated allowing for lifestage-specific evaluations from infant through adulthood, in the deterministic mode, the output accounts for human specific metabolism and physiology thus obviating the need for the inter-species extrapolation factor. The deterministic model can be used to simulate an "average individual" for all age groups (DAS *et al.*, 2011; Poet *et al.*, 2014; MRID 49074901).

Body weight can be calculated at any given age using a modified Gompertz growth function for both male and female (Smith *et al.*, 2014). Subsequently, tissue volumes, blood flows to tissue, metabolism rates, ChE enzyme activities, and exposure doses are calculated as a function of body weight for infants through adulthood, which in turn is also a function of age. Furthermore, the PBPK-PD model was refined to include probabilistic capabilities to allow for refining the intra-species extrapolation factor. The FIFRA SAP reviewed the initial multiple lifestage, probabilistic model at its February 2011 meeting and recommended some additional improvements (FIFRA SAP, 2011; Hinderliter *et al.*, 2011; Price *et al.*, 2011). In response to the SAP, DAS made multiple changes and performed additional analyses, including a global sensitivity analysis, improvements to the quantitative approach to evaluating population variability across individuals at a given age, and an uncertainty analysis on metabolism data from human hepatic microsomes and plasma to address variation in response that occurs from metabolism (Dow, 2014a, b). Code is available to simulate tissue dosimetry from dermal and inhalation exposure routes (Poet *et al.*, 2014).

To simulate exposures to infants and children, the model incorporates age-specific body weight prediction (Leucke *et al.* (2007) and Young *et al.*, (2009)), which is then used to scale the tissue volumes, blood flows to tissues, and ChE activities (Figure 4). In addition to age-related physiology, the model also incorporates age-specific metabolism based on *in vitro* data, allowing the model to assess changes in metabolism from infancy to adulthood. Total V_{max} of human enzymatic metabolism of CPF to TCPy, CPF to CPF-oxon, and CPF-oxon to TCPy in the liver over various ages were scaled to age-dependent volume of the liver (Table 4.8.3.1) and concentration of microsomes in the liver, which is approximately 37 mg/g tissue (Barter *et al.*, 2008). Thus, even in cases where the enzymatic measures show no *in vitro* age- or physiological-dependences (on a per mg protein basis), the total enzymatic capacity of the individual varies with age. In plasma, however, *in vitro* PON1 metabolism of CPF-oxon to TCPy was age-specific on a per ml plasma volume basis, as described previously. Therefore, the age-specific V_{max} was scaled to age-dependent volume of the blood, resulting in plasma metabolism varying by almost 2 orders of magnitude. Values and sources of model parameters can be found in Tables 4.8.3.1–4.8.3.2 and were extracted from Dow 2011 and MRID 49248201.

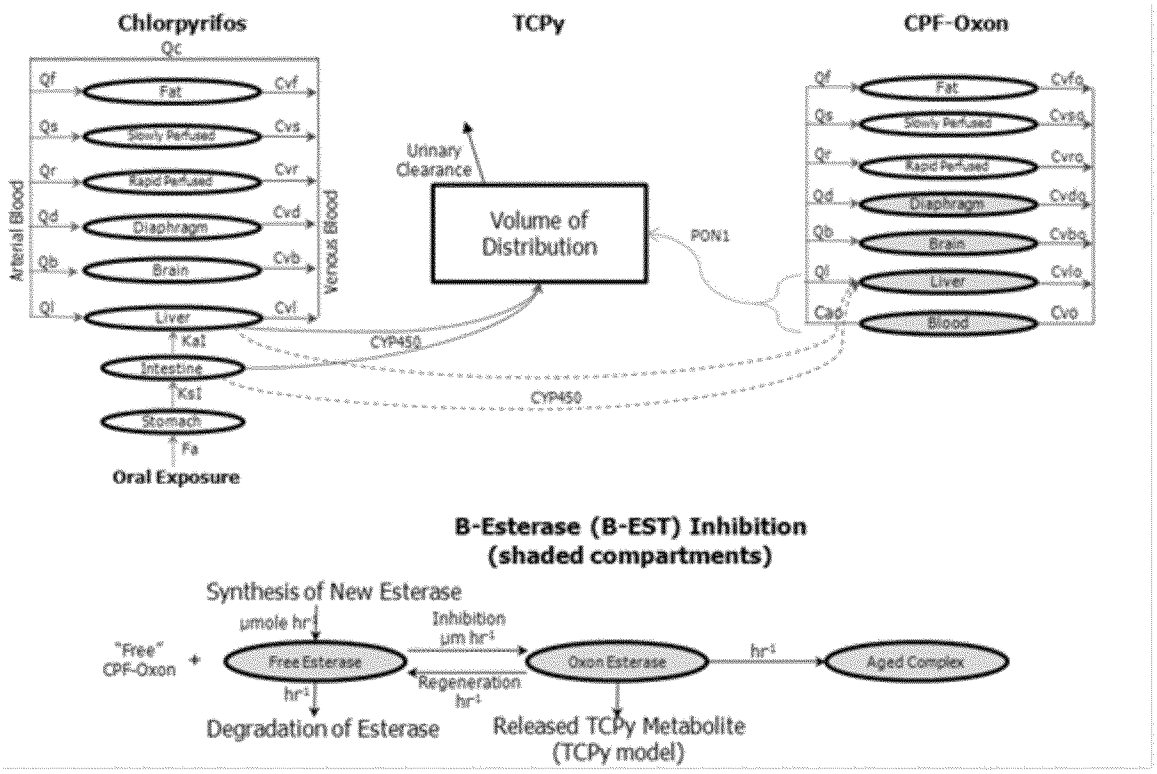


Figure A.2. PBPK/PD model (Typical Adult model) structure. The shaded compartments denote tissues which contain B-esterases (bottom panel). Tissue volumes and enzyme activities (V_{max}) change with age based on liver and/or blood compartmental growth (Extracted from Dow, 2011).

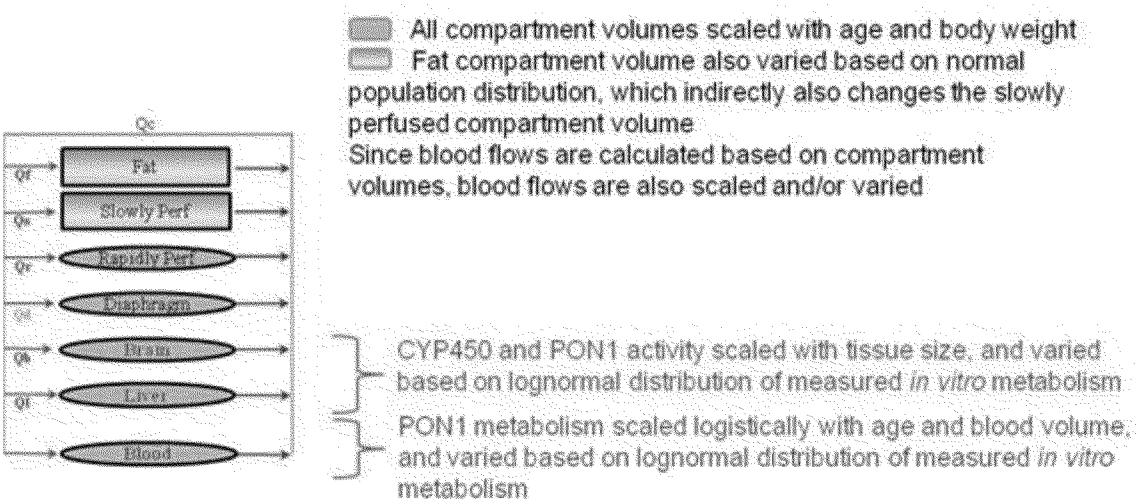


Figure 4. Schematic of age and body weight dependences in the PBPK-PD model. All compartment volumes and blood flows vary with age and body weight. *In vivo* metabolic rates are scaled based on tissue size (measured *in vitro* values scaled to describe tissue-specific (brain, blood, and liver) metabolism); in blood, PON1 metabolism of oxon is not only blood volume but also age-dependent (Extracted from Dow, 2011).

Table A.5.3.1. Pharmacokinetic model parameters.

Parameter	Value	Source
Tissue Ontogeny		
All	Scaled by age and body weight	See Table 4.8.3.2
Flows (L/hr/kg tissue volume)		
Cardiac Output	Summed from total tissue flow	See "*Blood flows*" in this section
Brain	30.6	Price *et al.*, 2003
Diaphragm	85.2	Luecke *et al.*, 2007
Fat	1.45	Luecke *et al.*, 2007, Cowles *et al.*, 1971, Price *et al.*, 2003
Liver	50.4	Price *et al.*, 2003
Rapidly Perfused	61.8	Adrenal/Spleen average: Price *et al.*, 2003
Slowly Perfused	1.80	Bone: Price *et al.*, 2003
Partition Coefficients for CPF (tissue:blood)		
Brain	16.5	Lowe *et al.*, 2009
Diaphragm	3.85	Lowe *et al.*, 2009
Fat	250	Lowe *et al.*, 2009
Liver	12.8	Lowe *et al.*, 2009
Rapidly Perfused	16.5	Lowe *et al.*, 2009
Slowly Perfused	3.85	Lowe *et al.*, 2009
Partition Coefficients for CPF-oxon (tissue:blood)		
Brain	5.6	Lowe *et al.*, 2009
Diaphragm	1.8	Lowe *et al.*, 2009
Fat	75	Lowe *et al.*, 2009
Liver	4.5	Lowe *et al.*, 2009
Rapidly Perfused	5.6	Lowe *et al.*, 2009
Slowly Perfused	1.8	Lowe *et al.*, 2009
Hepatic CYP Metabolic Constants (per tissue wt)		
CPF → TCPy Vmax (nmol/hr/kg)	1580	Measured *in vitro*, Median: See Table 4.8.3.2
CPF → TCPy Km (µM)	53.8	Measured *in vitro*, Median: See Table 4.8.3.2
CPF → Oxon Vmax (µmol/hr/kg)	689	Measured *in vitro*, Median: See Table 4.8.3.2
CPF → Oxon Km (µM)	85.8	Measured *in vitro*, Median: See Table 4.8.3.2
Intestinal CYP Metabolic Constants (per tissue wt)		
CPF → TCPy Vmax (µmol/hr/kg)	36.8	Poet *et al.*, 2003
CPF → TCPy Km (µM)	55	Poet *et al.*, 2003

Parameter	Value	Source
CPF → Oxon Vmax (µmol/hr/kg)	10.0	Poet *et al.*, 2003
CPF → Oxon Km (µM)	8.1	Poet *et al.*, 2003
Brain CYP Metabolic Constants (per tissue wt)		
CPF → TCPy Vmax (µmol/hr/kg)	3.85	Extrapolated: CPF metabolism/mechanism of action
CPF → TCPy Km (µM)	5.38	See Section 2: CPF metabolism/mechanism of action
CPF → Oxon Vmax (µmol/hr/kg)	0.91	Extrapolated: CPF metabolism/mechanism of action
CPF → Oxon Km (µM)	8.60	See CPF metabolism/mechanism of action
PON1 Metabolic Constants		
Plasma Vmax (µmol/hr/kg)	Logistic Fit	Measured *in vitro*, Median: See Table 4.8.3.2
Plasma Km (µM)	192	Measured *in vitro*, Median: See Table 4.8.3.2
Liver Vmax (µmol/hr/kg)	3902	Measured *in vitro*, Median: See Table 4.8.3.2
Liver Km (µM)	498	Measured *in vitro*, Median: See Table 4.8.3.2
Intestine Vmax (µmol/hr/mg)	246	Poet *et al.*, 2003
Intestine Km (µM)	328	Poet *et al.*, 2003
Oral Absorption		
Stomach →Intestine	0.5	Fitted: Nolan *et al.*, Timchalk *et al.*, 2002
Intestinal Absorption	0.2	Fitted: Nolan *et al.*, Timchalk *et al.*, 2002
Plasma Protein Binding (%)		
CPF	99	Lowe *et al.*, 2009
Oxon	99	Lowe *et al.*, 2009
TCPy Compartmental Model		
Vd (L)	$0.2 \times BW^{1.117}$	Fitted: Nolan *et al.*, 1984, Timchalk *et al.*, 2002
Ke (/hr)	0.013	Fitted: Nolan *et al.*, 1984, Timchalk *et al.*, 2002
Cholinesterase Degradation Rates (hr-1)		
Butyryl	0.004	Fitted (repeat dose data in rats: DOW unpublished)
Acetyl	0.01	Standardized, Timchalk *et al.*, 2002b
Bimolecular Inhibition Rate (µM hr^{-1})		
Butyryl	2000	Timchalk *et al.*, 2002
Acetyl	220	Kousba *et al.* (2007)

Parameter	Value	Source
Enzyme Turnover Rate (hr^{-1})		
Butyryl	$1.17x10^{7}$	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Acetyl	$3.66x10^{6}$	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Carboxyl	$1.086x10^{5}$	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Enzyme Turnover Rate (hr^{-1})		
Butyryl	11700000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Acetyl	3660000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Carboxyl	108600	Albers *et al.*, 2010
Enzyme Activity (µmol/kg/hr)		
Brain ACHE	440000	Hojring *et al.*, 1976
Diaphragm ACHE	77400	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Liver Carboxyl	1920000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b; Pope *et al.*, 2005
Plasma Carboxyl	NA	Li *et al.*, 2005
Brain Butyryl	46800	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Diaphragm Butyryl	26400	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Liver Butyryl	30000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Plasma Butyryl	263000	Sidell *et al.*, 1975
Enzyme Reactivation Rate (hr^{-1})		
Butyryl	0.0014	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Acetyl	0.014	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Carboxyl	0.014	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Enzyme Aging Rate (hr^{-1})		
Butyryl	0.0113	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Acetyl	0.0113	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Carboxyl	0.0113	Carr and Chambers, 1996, Timchalk *et al.*, 2002b

Table A.5.3.2. Compartmental Growth.

Compartment		Fraction of Body Weight Equation[1]							Source for LifeStage Parameters
	Eqn. Format $V0+V1\times BW+V2\times BW^2+V3\times BW^3+V4\times BW^4+V5\times BW^5+V6\times BW^6$								(BW: Body Weight in g)
		V0	V1	V2	V3	V4	V5	V6	
Blood	Leucke	$9.15e^{-2}$	$-8.59e^{-7}$	$1.25e^{-11}$	$-6.46e^{-17}$	-	-	-	Young *et al.* 2009
	Young	$8.97e^{-2}$	$-3.50e^{-7}$	$6.54e^{-13}$					
	LifeStage	$8.970e^{-2}$	$-3.500e^{-7}$	$6.540e^{-13}$	-	-	-	-	
Brain	Leucke	$1.19e^{-1}$	$-3.51e^{-6}$	$4.28\ e^{-11}$	$-1.82\ e^{-16}$	-	-		Fit to Valentin *et al.* 2002 (bwt≤70) & Young *et al.* 2009 data (body weight>70 kg) and extrapolated values based on Table 4A (Young *et al.* 2009)
	Young	$1.41e^{-1}$	$-5.54e^{-6}$	$9.30\ e^{-11}$	$-6.83e\ e^{-16}$	$1.80e^{-21}$			
	LifeStage	$1.216e^{-1}$	$-3.465e^{-6}$	$4.354e^{-11}$	$2.463e^{-16}$	$5.132e^{-22}$			
Diaphragm	Leucke	$3.000e^{-4}$	-	-	-	-	-	-	Luecke *et al.* 2007
	Young	NA	-	-	-	-	-	-	
	LifeStage	$3.000e^{-4}$	-	-	-	-	-	-	
Fat*									
Female	Leucke	$5.91e^{-2}$	$1.20e^{-5}$	$-5.80e^{-10}$	$1.12e^{-14}$	$-6.36e^{-20}$	-	-	Fit to Valentin *et al.* 2002 & Lafortuna *et al.* 2005 data and extrapolated data based on equation in Fig 1B (Lafortuna *et al.* 2005)
	Young	$1.84e^{-2}$	$-6.86e^{-6}$	$2.46e^{-10}$	$-2.11e^{-15}$	$7.58e^{-21}$	$-9.94e^{-27}$	-	
	LifeStage	$9.217e^{-02}$	$1.401e^{-05}$	$-6.787e^{-10}$	$1.540e^{-14}$	$-1.558e^{-19}$	$7.249e^{-25}$	$1.273e^{-30}$	

Compartment		Fraction of Body Weight Equation[1]							Source for LifeStage Parameters
Male	Leucke	$3.95e^{-2}$	$1.59e^{-5}$	$-6.99e^{-10}$	$1.09e^{-14}$	$-5.26e^{-20}$	-	-	Fit to Valentin *et al.* 2002 & Lafortuna *et al.* 2005 data and extrapolated data based on equation in Fig 1B (Lafortuna *et al.* 2005)
	Young	$1.61e^{-2}$	$-3.59e^{-6}$	$-8.28e^{-11}$	$-3.57e^{-16}$	$4.73e^{-22}$	-	-	
	LifeStage	$3.484e^{-2}$	$2.803e^{-5}$	$-1.422e^{-9}$	$2.892e^{-14}$	$-2.718e^{-19}$	$1.203e^{-24}$	$-2.036e^{-30}$	
Liver	Leucke	$3.49e^{-2}$	$-3.23e^{-7}$	$2.13e^{-12}$	-	-	-	-	Fit to Valentin *et al.* 2002 (body weight≤ 70) & Young *et al.* 2009 extrapolated values based on Table 4A
	Young	$4.25e^{-2}$	$-1.01e^{-6}$	$1.99e^{-11}$	$-1.66e^{-16}$	$4.83e^{-22}$	-	-	
	LifeStage	$3.917e^{-2}$	$-6.789e^{-7}$	$1.082e^{-11}$	$-7.393e^{-17}$	$1.701e^{-22}$	-	-	
Rapid									
Lung	Leucke	$1.67e^{-2}$	$-9.96e^{-8}$	$-1.09e^{-13}$	$1.13e^{-17}$	-	-	-	Young *et al.* 2009
	Young	$1.860e^{-2}$	$-4.550e^{-8}$	-	-	-	-	-	
	LifeStage	$1.860e^{-2}$	$-4.550e^{-8}$	-	-	-	-	-	
Kidney	Leucke	$7.31e^{-3}$	$-8.29e^{-8}$	$2.13e^{-12}$	-	-	-	-	Young *et al.* 2009
	Young	$7.26e^{-3}$	$-6.69e^{-8}$	$3.33e^{-13}$	-	-	-	-	
	LifeStage	$7.260e^{-3}$	$-6.690e^{-8}$	$3.330e^{-13}$	-	-	-	-	
Pancreas	Leucke	$1.17e^{-3}$	$-1.18e^{-8}$	$1.81e^{-13}$	-	-	-	-	Brown *et al.* 1997 and Young *et al.* 2009
	Young	$1.48e^{-3}$	-	-	-	-	-	-	
	LifeStage	$1.480e^{-3}$	-	-	-	-	-	-	
Spleen	Leucke	$3.05e^{-3}$	$-2.09e^{-8}$	$1.24e^{-13}$	-	-	-	-	Young *et al.* 2009
	Young	$3.12e^{-3}$	$5.57e^{-9}$	-	-	-	-	-	
	LifeStage	$3.120e^{-3}$	$5.570e^{-9}$	-	-	-	-	-	
GI	Leucke	$1.93e^{-2}$	$-4.42e^{-7}$	$9.28e^{-12}$	$-4.88e^{-17}$	-	-	-	Brown *et al.* 1997
	Young	NA	-	-	-	-	-	-	
	LifeStage	$1.650e^{-2}$	-	-	-	-	-	-	

Compartment		Fraction of Body Weight Equation[1]							Source for LifeStage Parameters
Slow									
Non-Fat adipose*	Leucke	NA	NA	NA	NA	NA	NA	NA	Fit to Valentin *et al.* 2002 & Lafortuna *et al.* 2005 fat data/0.8 (Valentin 2002) data and extrapolated data based on equation in Fig 1A (Lafortuna *et al.* 2005)
	Young	NA	NA	NA	NA	NA	NA	NA	
	LifeStage	$2.044e^{-1}$	$2.617e^{-5}$	$-1.542e^{-9}$	$3.268e^{-14}$	$-3.116e^{-19}$	$1.387e^{-24}$	$-2.35e^{-30}$	
Muscle	Leucke	$9.61e^{-2}$	$-4.88e^{-6}$	$3.05e^{-10}$	$-3.62e^{-15}$	$1.22e^{-20}$	-	-	Fit to Valentin *et al.* 2002 & Janssen *et al.* 2000 data and extrapolated values based on Fig 2A (Janssen *et al.* 2000)
	Young	$9.68e^{-1}$	$-3.32e^{-6}$	$1.83e^{-10}$	$-1.24e^{-15}$	-	-	-	
	LifeStage	$1.251e^{-1}$	$1.458e^{-5}$	$-2.927e^{-10}$	$2.114e^{-15}$	$-5.250e^{-21}$	-	-	
Skin	Leucke	$1.07e^{-1}$	$-3.26e^{-6}$	$6.11e^{-11}$	$-5.43e^{-16}$	$1.83e^{-21}$	-	-	Young *et al.* 2009
	Young	$1.03e^{-1}$	$-2.56e^{-6}$	$3.68e^{-11}$	$-2.580e^{-16}$	$8.620e^{-22}$	$-1.100e^{-27}$	-	
	LifeStage	$1.030e^{-1}$	$-2.560e^{-6}$	$3.680e^{-11}$	$-2.580e^{-16}$	$8.620e^{-22}$	$-1.100e^{-27}$	-	
Bone Marrow	Leucke	$5.19e^{-2}$	$8.06e^{-7}$	$-1.96e^{-10}$	$7.63e^{-15}$	$-1.08e^{-19}$	$5.14e^{-25}$	NA	Brown *et al.* 1997 (red only, yellow is in adipose)
	Young	NA	NA	NA	NA	NA	NA	NA	
	LifeStage	$2.100e^{-2}$	-	-	-	-	-	-	

As it typical of PBPK/PD modeling, parameters that have no measured values were calibrated to fit time course of blood/tissue concentration data. Following the calibration of the model, additional data not used for calibration were used to evaluate the model's capability to predict other datasets and/or scenarios (*e.g.*, different species, different body weight, different dose ranges, or different exposure durations). The PBPK-PD model for chlorpyrifos was calibrated and evaluated using rat oral data, and human oral and dermal data (Timchalk *et al.*, 2012; Nolan *et al.*, 1984; Poet *et al.*, 2014). The rat model was calibrated using the time course of cholinesterase inhibition in RBC and brain, as well as chlorpyrifos concentrations in plasma. The human model was calibrated using the time course of plasma concentrations of chlorpyrifos and TCPy, urine concentrations of TCPy, and cholinesterase inhibition in plasma. The model predictions agree better with blood chlorpyrifos and plasma cholinesterase data for oral exposure than dermal exposure (Poet *et al.*, 2014). For oral exposure, the model slightly over-predicted chlorpyrifos concentrations in blood, and predicted plasma cholinesterase levels agreed with data. For dermal exposure, the model was optimized by fitting to the TCPy data (Nolan 1984) following a 24-hour dermal exposure. The model over-predicted the chlorpyrifos concentrations in blood by five to seven fold between 6 to 24 hours, as well as the inhibition of plasma cholinesterase. These results suggested that some additional loss of chlorpyrifos following dermal exposure may be possible and not described in the current version of the model. This additional loss, however, was not likely to oxon because of the over-prediction of plasma inhibition. The model-predicted blood concentration of chlorpyrifos, however, agreed with the dermal data at earlier time point (at 2 hour) and after the termination of exposure (at 48 hour).

Appendix 5.0: Verification of the lower bound of 90% CI of the estimated slope could be used to estimate the 95% lower limit of estimated concentration of CPF in cord blood

Mathematical derivation

Let ΔX_{true} be the true change in X given a ΔY change in Y of a general form of simple linear regression Y = intercept + b*X + ε. The ΔX_{true} can be calculated as $\Delta X_{true} = \Delta Y/b_{true}$, where b_{true} is the true slope.

If b_l is the 95% lower confidence limit (one-sided) of a slope b estimated from a set of data, then, there is a 95% confidence that the true slope b_{true} is greater than b_l. Therefore, we have 95% confidence that $\Delta X_{true} = \Delta Y/b_{true}$ is greater than $\Delta Y/b_l$ if ΔY is negative.

Simulation

The 95% lower confidence limit of an estimated ΔX associated with a ΔY as described above and presented in Table 2. Predicted levels of food exposure to chlorpyrifos from the 2014 revised risk assessment (Drew, 2014) and associated maximum and 24 hour blood concentrations in adult females was also verified by a simulation. The following is the (defined) equation of a true relationship between X and Y:

$$Y = 4.6 - 0.006*X + \varepsilon,$$

where $\varepsilon \sim N(0, 0.1547)$ and X follows lognormal distribution with arithmetic mean = 3.17 and standard deviation = 4.61. The true value ΔX_{true} associated with an arbitrary reduction ΔY = -0.05 is calculated as -0.05/(-0.006). For this simulation, a sample of 265 data points with the relationship described by Rauh *et al.* (2011) were randomly generated and the slope and its 95% lower confidence limit (one-sided) was estimated. The ΔX_L = -0.05/(95% lower confidence limit of the slope) was compared to the true value ΔX_{true}. The simulation was set to have 10,000 iterations (*i.e.* 10,000 samples of 265 data points per sample) with the proportion of iterations that $\Delta X_L < \Delta X_{true}$ computed. If the proportion of iterations that $\Delta X_L < \Delta X_{true}$ is about 95%, then the logic behind calculating the 95% lower limit of an estimated ΔX associated with a ΔY using 95% lower confidence limit (one-sided) of the estimated slope is verified. (Note that some statistics from Rauh's 2011 study were used as parameters of the simulation. Note that the parameters of simulation (*i.e.* intercept, slope, ε, range of X, ΔY, etc.) are arbitrary can be changed to any other values and the conclusion reached by the simulation is expected to be the same.

Result: the proportion of iterations that $\Delta X_L < \Delta X_{true}$ is 95.3% which is close to the target proportion of 95%. Therefore, the calculation used to derive the results presented in **Table 2** of a 95% lower limit of an estimated ΔX associated with a given ΔY using the 95% lower confidence limit is verified.

SAS code for simulation

```
%Macro DoseLevel(N =,     /* number of subjects in sample*/
                          NSim=,        /* number of iterations in simulation */
                          AME =,        /* arithmetic mean of exposure in old study */
                          ASDE =,       /* standard deviation of exposure (actual scale) of
old study */
                          int =,   /* intercept of regression line model of log-transformed Y
*/
                          slope=,/* effects per unit exposure, i.e. slope of exposure */
                          SD0=, /* standard deviation of Y at zero exposure */
                          YDiff=,       /* amount change in Y */
                          seed=);

        *==> in log-transformed exposure data;
        %let SDE = sqrt(log((&ASDE/&AME)**2 + 1));
        %let ME = log(&AME) - 0.5*&SDE**2;

        Proc datasets nolist; delete simmer results power; quit;

        Data Simmer;

                call streaminit(&seed);

                do Sim = 1 to &NSim;
                        do sub = 1 to &N;
                                *==> create random value to derive exposure of each subject;
                                random=rannor(&seed);

                                *==> fix the random value if its value too high;
                                if random > 3.1 then random = 3.1 + 0.1*rand('uniform');

                                *==> compute the exposure for each subject;
                                CPF = exp(&ME + random*&SDE);

                                logWM = &int + &slope*CPF + rannor(&seed)*&SD0;
                                output;

                        end; *sub;
                end; *Sim;
        run;
```

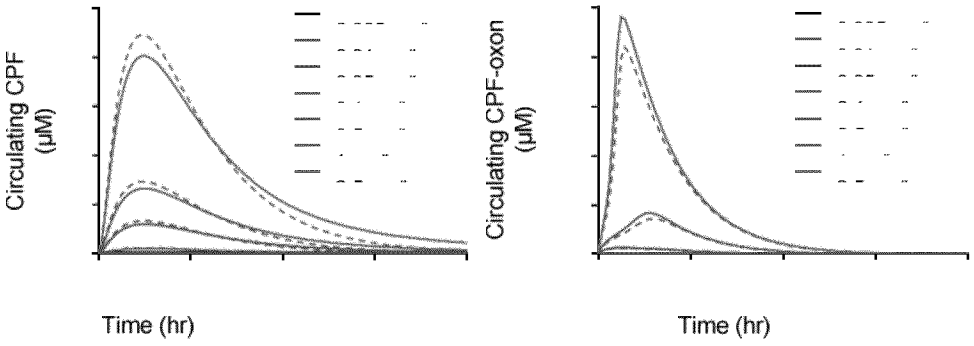
```
        ods select none;
        Proc glm data = Simmer ;
                by Sim;
                model logWM = CPF/solution clparm alpha=0.1;
                ods output ParameterEstimates = Parm;
        run;

        Data Parm;
                set Parm;
                if Parameter = "CPF";
                TrueCPF  = &YDiff/&Slope;
                CPF_LowerCL = &YDiff/LowerCL;
                check_CPF_LB = (CPF_LowerCL < TrueCPF);
        run;

        ods select default;
        Proc means data = Parm mean;
                var check_CPF_LB ;
        run;

%Mend;
%DoseLevel(N = 265,                     /* number of subjects in sample*/
                NSim=10000,             /* number of iterations in simulation */
                AME = 3.17,             /* arithmetic mean of exposure in old study */
                ASDE = 4.61,            /* standard deviation of exposure (actual scale) of old study */
                int = 4.6,        /* intercept of regression line model of log-transformed Y */
                slope= -0.006,          /* effects per unit exposure, i.e. slope of exposure */
                SD0= 0.1547,            /* standard deviation of Y at zero exposure */
                YDiff= -0.05,           /* amount change in Y */
                seed=2546);
```

Appendix 6.0: Selected Figures from Poet (2015) Multi-Route, Lifestage, and Pregnancy PBPK/PD model for Chlorpyrifos and Chlorpyrifos-Oxon Submitted to EPA



Figures A.3 and A.4. Predicted chlorpyrifos (A) (Figure A.3) and chlorpyrifos oxon (B) (Figure A.4) in blood following oral doses from 0.005 to 2.5 mg/kg in pregnant (solid lines) and non-pregnant (dashed line) women.

Appendix 7.0: Comparative analysis of NRC evaluation of methyl mercury epidemiology studies compared with chlorpyrifos and OP studies

For methyl mercury, there are three epidemiological studies considered for quantitative analyses in the IRIS assessment. These longitudinal, developmental studies were conducted in the Seychelles Islands, the Faroe Islands, and New Zealand. The subjects of the Seychelles longitudinal prospective study were 779 mother-infant pairs from a fish-eating population (Myers *et al.*, 1995a-c, 1997; Davidson *et al.*, 1995, 1998). Infants were followed from birth to 5.5 years of age, and assessed at various ages on a number of standardized neuropsychological endpoints. The Faroe Islands study was a longitudinal study of about 900 mother-infant pairs (Grandjean *et al.*, 1997). The main independent variable was cord-blood mercury; maternal-hair mercury was also measured. The New Zealand study was a prospective study in which 38 children of mothers with hair mercury levels during pregnancy greater than 6 ppm were matched with children whose mothers had lower hair mercury levels (Kjellstrom *et al.*, 1986, 1989). The Seychelles study yielded little evidence of impairment related to *in utero* methylmercury exposure, whereas the other two studies found dose-related effects on a number of neuropsychological endpoints.

The methyl mercury RfD is based on a benchmark dose analysis using epidemiology studies on different neuropsychological effects in the offspring of exposed mothers at 7 years of age (Grandjean *et al.*, 1997; Budtz-Jørgensen *et al.*, 1999a). At 7 years of age, children were tested on a variety of tasks designed to assess function in specific behavioral domains such as the Continuous Performance Test, Boston Naming Test, and California Verbal Learning Test. The cutoff for abnormal response was set at the lowest 5% (5^{th} percentile) of children. Generally speaking, children who function at or below approximately the 5^{th} percentile are considered significantly developmentally compromised for the ability that is being measured. A BMR of 0.05 was used in the methyl mercury IRIS assessment, which would result in a doubling of the number of children with a response at the 5^{th} percentile of the population.

Table A.7.1: Comparison of the major considerations from NRC for methyl mercury and with the chlorpyrifos studies	
2000 NRC Report on Methyl mercury	Chlorpyrifos & CCCEH study
Susceptible subpopulations	
Interindividual toxicokinetic variability; Development of the RfD must consider this individual variation; in particular, any biomarker-based measure should account for the toxicokinetic variability in the population.	Similar to methyl mercury, TK variability needs to be considered.
Toxicodynamic variability	Chlorpyrifos has similar uncertainty as methyl mercury: AOP is not known. Thus, TD variability is unknown & cannot be quantified
Nutritional deficits	The CCCEH cohort is made up of urban, low income population. The nutritional status is unknown in the CCCEH mothers.
Measures of exposure	
Lack of dietary-intake data in methyl mercury studies	Dermal exposure expected to be primary exposure route from indoor, residential use. In the CCCEH studies, there is a lack of key exposure information: timing & frequency of applications; amount applied; and surface wipe samples in the CCCEH studies. However, EPA has developed scenarios to make predictions about dermal exposure and associated internal dose. The agency's analysis reasonably approximates the indoor exposure to chlorpyrifos prior to the voluntary cancelation. Thus, there remains uncertainty about actual exposure to the mothers, the agency has reasonable estimates of such exposure.
Extrapolation from biomarker Hg content to MeHg intake	
Nutritional and dietary confounders and effect modifiers (from exposure to fish)	Not applicable

Table A.7.1: Comparison of the major considerations from NRC for methyl mercury and with the chlorpyrifos studies	
2000 NRC Report on Methyl mercury	Chlorpyrifos & CCCEH study
Co-exposure to other neurotoxicants (e.g., PCBs)	The CCCEH authors were able to measure and model important environmental exposures including environmental tobacco smoke (ETS), polyaromatic hydrocarbons (PAHs), methylmercury and other ChE-inhibiting pesticides such as diazinon and propoxur (Whyatt & Rauh, 2011). Given the known relation between both lead and also methyl mercury and neurodevelopment, the authors performed supplemental analyses. The results of these analyses showed that neither pre-natal nor post-natal blood lead levels or methyl mercury levels measured in cord blood were significantly correlated with chlorpyrifos, and was therefore not considered a confounding variable in the association of interest within the Columbia cohort (Whyatt & Rauh, 2011; Rauh *et al*., 2006). This additional work significantly reduces uncertainty in the CCCEH research results. CCCEH researchers have also evaluated the association between exposure to the pyrethroid permethrin and the pyrethroid synergist piperonyl butoxide (PBO) and neurodevelopmental outcomes (Horton *et al*., 2011), and observed no statistically significant associations with permethrin. The statistically significant associations with PBO were found to be independent of those observed with chlorpyrifos. In addition, it is noted that the CHAMACOS study authors recently assessed the relationship between prenatal and postnatal polybrominated diphenyl ethers (PBDE) exposure and neurodevelopmental outcomes (Eskenazi *et al*., 2013). They observed associations between PBDE exposure and neurodevelopmental outcomes, but concluded that these associations were independent of those previously observed in the CHAMACOS cohort between OP exposure and child neurobehavioral development (Bouchard *et al*., 2011; Eskenazi *et al*., 2007; Marks *et al*., 2010).

Table A.7.1: Comparison of the major considerations from NRC for methyl mercury and with the chlorpyrifos studies	
2000 NRC Report on Methyl mercury	Chlorpyrifos & CCCEH study
Co-exposure to other forms of Hg	As shown in Whyatt *et al.* (2003), cord blood samples from CCCEH mothers included other OPs (diazinon) and other potent AChE inhibiting pesticides such as propoxur and carbofuran. The impact of co-exposure to AChE inhibiting pesticides is not clear.
Inability to measure peak exposures: Measurement of cord blood does not detect temporal variability in exposure and reflects exposure during a period late in gestation. Therefore cord-blood concentrations might not correspond to the periods of greatest fetal sensitivity to Hg neurotoxicity	Chlorpyrifos has similar uncertainties as methyl mercury. The temporal nature of exposure is unknown but has been predicted based on best available information. Without an AOP for neurodevelopmental effects, critical period(s) of exposure during development are not known. Therefore cord-blood concentrations may or may not correspond to the periods of greatest sensitivity to chlorpyrifos
Temporal matching of exposure to critical periods of susceptibility for the developing fetal brain: uncertainty in the linkage between the time and the intensity of exposure to critical periods of brain development.	
Lack of consideration of other key or most-sensitive health end points	
Potential cardiovascular or immune-system effects: Neurodevelopmental effects are the most extensively studied sensitive end point for MeHg exposure, but there remains some uncertainty about the possibility of other health effects at low levels of exposure. In particular, there are indications of immune and cardiovascular effects, as well as neurological effects emerging later in life, that have not been adequately studied.	Across the CCCEH study, other endpoints also being evaluated (obesity, asthma, etc). Among OP studies, CHAMACOS has shown effects on heart & lung function but using childhood biomarkers, not in utero. A study in Ecuador shows autonomic nervous system impacts (i.e. heart rate and blood pressure) but at potentially cholinergic toxic internal dose levels (Suarez-Lopez et al, 2012; 2013; 2013a). Given the number of parameters begin measured by CCCEH researchers, this is unlikely to be a substantial uncertainty.
Neurological sequelae (*i.e.*, late-emerging effects)	CCCEH investigators are making observations on the children across time (birth to 11 years old). Motor skill deficits observed in Rauh *et al.* (2006) appear to continue in Rauh *et al.* (2015) with hand tremors while writing.